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Towards a sustainable approach for development of biodiesel from plant and microalgae

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ABSTRACT

The production of biodiesel can be accomplished using a variety of feedstock sources. Plant and microalgae based feedstocks are prominent and are studied extensively. Plant based feedstocks cultivated as monoculture on wastelands and trees in forests can cater towards partial fulfillment of feedstock requirements for biodiesel industry. Synthesis of biodiesel from microalgal oil has gathered immense interest and has potential to cater to the increasing feedstocks demands of the biodiesel industry. The major advantage offered by microalgal oil, as compared to plant based oils, is its potential for culture on non-arable land. Despite of the advantages of microalgal oil as a feedstock for biodiesel, there are constraints that have to be overcome in order to make it economical and sustainable. Sustainable approaches for both the plant and microalgae as feedstocks have been drawn. Despite there being several plant species, few have been found to be desirable as feedstocks for biodiesel production based on their lipid profiles. Among the microalgae, there are thousands of species and several of these have been cultured for extracting the oil to explore their feasibility in utilization as biodiesel feedstocks. Though, several of the microalgal species have shown potential for high biomass growth and lipid productivity, only a few have been found to provide a high biodiesel yield and conversion. Due to the several steps involved in the extraction of oil which are energy intensive, the cost of biodiesel from microalgal oil is high as compared with that obtained from the plant oils. A sustainable approach for utilizing plant and microalgal oils as feedstocks for biodiesel have been discussed. The emerging cost effective methods in production of biodiesel have been described. The energy return and greenhouse gas emissions from biodiesel have been outlined. Together, the plant oil and microalgal oil can offer potential source of feedstocks for the production of biodiesel.

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1. Introduction

The world's anticipated energy requirement for 2030 shall be 50% more than it is today. The transportation sector alone accounts for 30% of the world's total energy consumption and the last three decades have witnessed a steep rise in the number of transportation vehicles worldwide, most of which are private car [1]. The global energy demand in the transportation sector is expected to grow by 2% per year. This would lead to 80% higher emissions in terms of greenhouse gas emissions and energy use in comparison to that of 2002 levels [2]. The combustion of diesel and gasoline derived from crude oil in compression ignition (CI) and spark ignition (SI) vehicles emits carbon dioxide along with hydrocarbons, particulate matter, carbon monoxide, oxides of nitrogen, and other carcinogens that pose direct and indirect dangers to mankind and ecosystems. Worldwide concern regarding the increase in carbon dioxide and other pollutants in the atmosphere as well as the dwindling reserves of crude oil have led to the development of renewable fuels. Despite the differences in opinion on the amount of crude oil available, its exhaustion in coming years seems inevitable. The renewable sources of energy including solar, wind, ocean, hydropower, geothermal, and biomass have the capability to provide alternative sources of clean energy [3]. Baring biomass and solar energy, these renewable energy sources are place specific and hence can be utilized only at locations that are suitable for their harnessing. Another source of renewable fuel that has received attention worldwide due to its compatibility as a fuel in a compression ignition (CI) engine is biodiesel. In 2008, the global production of biodiesel was 12 billion litres increasing to 17 and 19 billion litres in 2009 and 2010, respectively [4].

Biodiesel is produced from a wide variety of raw materials (comprising of triglycerides as major component) by either esterification or transesterification or both depending on the acid value of the feedstock and the type of catalyst (acid or alkaline) used for its synthesis. A feedstock in the terminology of biodiesel has been well defined by Sun et al. [5] as "the raw material from which oils and fats are derived". Biodiesel has been blended with mineral diesel (up to 20%) and has been used as fuel in CI engines, possessing a comparable calorific value to mineral diesel. As biodiesel is compatible with mineral diesel, it can even be used in neat form thus proving an alternate to mineral diesel with few, if any, modifications in CI engines [6]. Biodiesel emits lower exhaust emissions on its combustion as compared to mineral diesel and have shown a comparable performance as that with mineral diesel and aviation fuel [7]. Owing to its renewability and sustainability, the production of biodiesel has gradually increased in recent years.

The wide spectrum of feedstocks that can be used to synthesize biodiesel are edible oils (usually wherever in surplus), non-edible oils, waste cooking oils, waste frying oils, animal or fish waste oil and microalgae oil [8,9]. Approximately 7% of edible vegetable oils globally have been reported to be diverted to the production of biodiesel in 2007 [10]. Borugadda and Goud [11] reports that among the plant origin oils, edible oil contributes 95% of the feedstock constituent for the production of biodiesel. The advantage of edible oil as a feedstock for production of biodiesel is their low free fatty acid (FFA) content (i.e. $>1\%$) which allow single step transesterification of the oil [12]. Edible oil feedstocks that are used worldwide in synthesis of biodiesel are soybean, rapeseed, palm, and sunflower [13]. However, with the shrinking arable land availability and the worldwide concern over the fuel versus food debate, it does not seem justified to divert edible oil to the production of biofuels. The limited availability of the edible oil in many developing and undeveloped nations pose a question mark to the continuance of production of biodiesel from edible oils. Non-edible oils, waste cooking/frying oil, animal fats and microalgal oil contain a high FFA ($>4\%$) content which has to undergo acid esterification so that they do not saponify as with the alkali catalysts. Several solid acid catalysts have been developed and used for the simultaneous esterification and transesterification of the FFA and triglycerides present in oil/fat in the feedstock. Exploring the possibilities of the non-edible feedstocks, Balat [10] reported on the feasibility of a few non-edible plant species to be efficient in the production of biodiesel. These include *Jatropha curcas*, *Pongamia pinnata*, *Madhuca indica*, and several other plant species along with the microalgal oil. Singh [14] has also reported on the feasibility of *P. pinnata*, and *M. indica* along with a novel feedstock, *Schleichera triguga* to have a high potential for conversion to biodiesel with a high yield. The advantage of the plant and microalgal based feedstocks is that they are diverse (in terms of species and strains). So, if one of the feedstocks is scanty, the demand can be fulfilled by an alternative feedstock. However, there is a disadvantage with the usage of some of these feedstocks, as at least a few of them have alternate applications in medicine, soap manufacture and other useful applications. These plant derived oils also have a low energy density and are not economical in present scenario as compared to mineral diesel. The non-edible oils utilized in the pharmaceutical and soap industries

and have other applications [15]. The waste cooking/frying oil and waste animal/fish oil are too scanty to support a large or medium scale biodiesel plant. Algae as a feedstock for biodiesel are in the preliminary stages of research but have shown immense potential to provide alternative and a new generation feedstock. The lipid productivity of microalgae with respect to the dry weight of biomass can be 15 to 300 times greater than that derived from plants [16]. However, their cultivation and extraction of oil along with optimization of the important parameters (light, pH, temperature, nutrients) for a high yield of oil with low cost is presently a challenge.

The transesterification reaction for the synthesis of biodiesel involves reaction of oil or fat as triglycerides with a lower group alcohol (preferably methanol or ethanol due to their high reactivity owing to shorter chain length) in presence of an acid or alkali catalyst. Esterification is the process adopted for the conversion of FFA content in oil/fats to fatty acid alkyl ester (FAAE) in presence of an acid catalyst. Hence, with feedstock containing high FFA content, a two step process comprising of esterification followed by transesterification is usually adopted [17]. The preference of methanol over ethanol as alcohol is due to the higher reactivity and lower cost of the former. To enhance the rate of reaction, a catalyst (homogeneous or heterogeneous) is compulsorily added. The only case where the catalyst is usually not added is when the esterification/transesterification reaction is done at supercritical conditions [15]. A supercritical method is the technique by which, at a high temperature and pressure, the dielectric constant of the reactant (alcohol in the present case) is reduced to facilitate the formation of a single phase as compared to the usual two phases of oil and alcohol. The advantage with supercritical method is that the purification or separation steps are not required [18,19]. However, the process requires high energy consumption due to high temperature requirement and is not economically feasible.

The product obtained after transesterification of oil or fat primarily comprises of FAAE and is termed as ‘biodiesel’. A useful co-product ‘glycerol’ is also obtained which has numerous industrial applications in food, cosmetic, and pharmaceutical industries [19]. After separation, glycerol is purified for its usage in industrial applications. The biodiesel is purified separately to remove the unreacted components and by-products that are formed during the reaction viz. soap, residual alcohol, glycerol, tri-, di-, monoglycerides, water, and catalyst.

The high price of biodiesel is attributed to the limited availability of the feedstocks (edible/non-edible oil) which have other useful applications (medicine, soap manufacture, human consumption) and limited availability of arable land for cultivation of biofuel crops. The only feedstocks that does not have alternate application or very limited application is the waste cooking, frying oil, waste animal and fish fats but their availability to support large demand to feedstock is not documented at present and is scanty to support a biofuel industry. The feedstock thus plays an important role in the synthesis of biodiesel. At present, plant oils (edible and non-edible) have been extensively used for the production of biodiesel because of their easy availability and high saponification value that enables transesterification. Recently, the alternative feedstocks viz. animal fats, waste cooking/frying oil, and oil from microbial species have also been explored for synthesis of biodiesel [20]. The future of biodiesel as a prominent transport fuel lies in the easy of availability of the feedstock. Animal fat and waste cooking/frying oil has limited availability and hence the focus is on the plant and microalgae based oil. This review deals with the various aspects of microalgae and plant oils for the synthesis of biodiesel and discusses the potential of plant and microalgae based feedstocks for the production of biodiesel and the recent approaches to their sustainability. The present review also deals with the extraction of lipids, conversion by transesterification, effect of feedstock lipids on fuel properties. Limitations and constraints of microalgal derived biodiesel along with its advantages and the possible measures to overcome the limitations have been explored. The present article firstly deals with various aspects of microalgal and plant oil like their pros and cons, characteristics as feedstock oil, extraction and conversion technologies and the effect on biodiesel properties as well as its emissions. The following sections elaborate the sustainable and emerging cost effective technologies in the biodiesel field. The final section discusses energy return and greenhouse gas emissions from biodiesel as well as plant oils and microalgal oils: a complementary to each other.

2. Microalgal and plant oil for synthesis of biodiesel

A constant supply of the feedstock is a major challenge for the commercialization of biodiesel. The feedstock is also a major contributor towards the total production cost of biodiesel. The overall cost of the feedstock may vary depending on the availability and alternative uses. Ahmad et al. [21] estimated the contribution of feedstock in production cost of biodiesel to be 75%. Demirbas [22] reported the feedstock to contribute 80% of the total production cost, whereas, Baroi et al. [23] reported the contribution of feedstock to be 88% of the total production cost of biodiesel. Thus, feedstock selection and strategies to utilize a low cost feedstock production may be a step towards the economical production of biodiesel. Biodiesel feedstocks are categorized as first, second and third generation. The first generation feedstocks comprise of edible plant oils. The second-generation feedstocks include non-edible plant oils, and waste cooking/frying oil. The oil derived from algae and other microorganisms are considered to be third generation feedstocks.

The current potential feedstocks for the synthesis of biodiesel include plant and microalgal oils. Among the plants and trees, several species bear fruit and seed containing oil and thus have potential to provide feedstocks that can be used for synthesis of biodiesel. The advantage of plant oils is that they are easily available worldwide and have a short carbon cycle period of few years as compared to mineral fuels that take millions of years for their formation [24]. There have been plantations of non-edible plant species on waste lands and marginal land in several countries viz. India. However, there is no data on the organic carbon the plant based feedstock (viz. *J. curcas*) takes from the soil. This is not an issue with the algae as they are grown in aquatic environment.

The plant feedstocks comprise of triglycerides, free fatty acids and other constituents in minor quantities (phorbol esters in *J. curcas*, saponins in *M. indica*; cyanolipids in *S. triguga*; and furanoflavones, furanoflavonols, chromenoflavones, flavones, and furanodiketones in *P. pinnata*). The presence of these constituents makes these oils non-edible for human consumption [13,25]. These lipids may be in form of mono-, di-, or tri-glyceride or free fatty acids and have varying affinity for their conversion to biodiesel [26]. Triglycerides are considered to be the best substrate to produce biodiesel [20,27]. Hence, both lipid content and the quality of the lipid are important in selection of a feedstock for the synthesis of biodiesel [28]. The selection of a feedstock for the synthesis of biodiesel determines the number of carbon atoms per molecule and the structure of bond in the molecule. The number and type of bonds present in the carbon chain then determine the fuel properties of biodiesel [5].

Among the plants, new species have continuously been explored for their oil yielding efficiency and feasibility to convert it to biodiesel. These includes *S. triguga* (kusum), *Phoenix dactylifera (*date seed), *Tenebrio molitor* (yellow mealworm beetle), *Datura stramonium* L., *Guizotia abyssinica* L.(niger oil), *Terminalia belerica* Roxb., *Zanthoxylum bungeanum*, *Haematococcus pluvialis*,

Xylocarpus moluccensis [29–36]. Table 1 depicts the list of recently explored feedstocks for biodiesel production. Most of these feedstocks have shown high conversion rate (> 96.5%) thus fulfilling the EN 14103 specifications. These feedstocks are non-edible, though many have alternative applications. The plant, *J. curcas* is a potential feedstock for biodiesel because it can grow on marginal soils, drought resistant nature and to some extent is pest resistant. *J. curcas* has a range of characteristics for coping with water stress. The plant combines C3-/CAM photosynthesis in succulent stems where water is stored. The leaves of the plant shift from C3-metabolism to CAM for an increased efficiency in the use of water [37]. This has led to cultivation of *Jatropha* in India, China, South America, and Africa [38]. Grass et al. [39] reported that for the cultivation of 1000 kg of *Jatropha* seeds requires 40 kg nitrogen, 16 kg phosphorous, and 10 kg potassium to maintain soil fertility. However, when subjected to stressed conditions (low nutrient and low water conditions), the oil yield is considerably reduced. Hence, the monoculture of *J. curcas* has become debatable in recent times. Research into *J. curcas* as a dedicated oilseed energy crop through modern techniques such as transcriptomics and near infrared spectroscopy for trait identification is ongoing [40]. Another group of the feedstock that hold potential is yellow grease. Though, not widely explored, yellow grease holds significant potential towards production as a biodiesel feedstock.

The edible plants oils comprise predominantly of triglycerides with trace amounts of free fatty acids (FFA), whereas, the non-edible plant oils contains significant amounts of FFA (up to 20%) in addition to triglycerides. Presence of FFA in oil imparts a high acid value [41]. In general, the biodiesel production from refined oils is easier than the unrefined oils due to lower FFA content [42]. Refined oils contain an amount of FFA (usually < 3%), whereas used cooking oil, and animal fats contain between 2–7 and 5–30% FFA, respectively. High acid value feedstocks include jatropha oil (14%), rubber oil (17%), mahua oil (19%), polanga oil (22%), and tobacco oil (35%) [15]. Due to the limited availability of non-edible feedstocks and their alternative applications, a diversification of the feedstock has been advocated for synthesis of biodiesel by Karmakar et al. [43].

In comparison to plant oils, microalgae have even a shorter carbon period of only a few days as they grow very rapidly. The photosynthetic efficiency of microalgae exceeds that of the plants thereby making it a better prospect for the production of oil or other constituents. The photosynthetic efficiency of microalgae ranges from 3 to 8% as compared to 0.5% for terrestrial plants [44]. Many species of algae are able to convert sunlight, nutrients, and CO_2 into proteins, carbohydrates, and lipids with high growth rates that double their biomass up to five times in a day. Microalgae can be harvested daily and serve as a feedstock for biofuels, food (edible oil, nutraceuticals), fish feeds and chemicals [28]. Microalgae can be used as a source of feedstocks for the production of biodiesel, methane, ethanol, butanol, and hydrogen depending on the constituents of the microalgae (starch, sugar, or oil) [45]. The lipid portion of microalgae can be diverted for biodiesel production, whereas, the sugar comprises primarily of cellulose can be hydrolyzed and fermented to bioethanol [46]. When the microalgae are subjected to alteration in culture conditions viz. limited supply of nitrogen, high salinity, high light intensity; they start to accumulate lipids in high concentration in their cells. Microalgae are thus considered as a potential feedstock for the synthesis of biodiesel due to their efficiency to store high lipid content in their cells [20]. The exact reason for the oil production in microalgae is still not known, though a few hypotheses have been suggested by researchers. One of the hypotheses for storage of oil in microalgae is to provide buoyancy and to keep it afloat in water. However, when they are depleted of nitrogen and starts accumulating oil, some of the species sinks instead to enhanced buoyancy thus

Table 1
Novel feedstocks used for biodiesel production.

Feedstock	Found	Oil content (%)	Specific treatment	Production potential	Biodiesel yield (Y)/ conversion (C) (wt%)	Reference
Schleichera triguga (Kusum)	India, Myanmar, Sri Lanka, Timor, Java	–	Pretreatment: Hydrolysis of cyano-glucosides using zinc oxide and sodium bisulfate under pressure followed by settling and removal of aqueous layer	–	Y=95	[29]
Phoenix dactylifera (Date seed)	Egypt, Iran, Saudi Arabia, U.A.E., Pakistan, Algeria, Sudan, Iraq, Oman, Libya	5.0 to 13.2	–	7.85 million tons of date fruits in 2010	Y=98	[30]
Tenebrio molitor (Yellow mealworm Beetle)	Distributed all over the world	0.7 (fat & grease)	–		C=96.8	31
Datura stramonium L.	Warm & temperate regions in 100 countries	21.4	–	–	C=98	[32]
Guizotia abyssinica L. (Niger oil)	Ethiopia, India	30	–	200–300 kg ha^{-1} & 500–600 kg ha^{-1} (with good management)	C=98.7	[33]
Terminalia belerica Roxb.	India (Deciduous Forest)	31 (SD=0.31%)	–	–	C=98.5	[34]
Zanthoxylum bungeanum	China	27–31	–	C > 96	35	
Xylocarpus moluccensis	Indonesia	10.65–11.09	–	–	–	[36]

SD=Standard deviation.

defying the hypothesis. The other hypothesis is that the lipid acts as a reservoir for its long term survival when subjected to poor environmental conditions, while the polysaccharides cater the short-term energy needs [47]. The prospects of microalgae in development of biodiesel is not very recent and dates to 1978 when the US Department of Energy started a program named "Aquatic Species Program—Biodiesel from Algae" which focused on the surveying of microalgal species for the identification of suitable strains for their culture in outdoor open system [48]. The microalgae can be grown in either open raceway ponds or photobioreactors [49]. As the cost is one of the constraints in the commercialization of microalgae derived biodiesel, there is a debate over using the mode of cultivation i.e. either open raceway ponds or photobioreactors. Both the modes of cultivation offer advantages over the other in some way and have limitations too. While, raceway ponds are low cost in terms of capital as well as operating costs. The photobioreactor bears a high cost, but it can be better controlled due to the close system and offers a higher yield and productivity of microalgae. In a raceway pond, the algae, water, and nutrients circulate around a racetrack shaped pond with paddlewheels and are kept in constant flow. Raceway ponds are shallow to allow a better penetration of sunlight. Water and nutrients are constantly fed in a raceway pond and the mature algae that are suspended in water are removed from the other end [49]. The energy demand and plant complexity is considerably lower in raceway pond as compared to photobioreactors. The commercial raceway ponds are elliptical in shape with depth of 15–30 cm and the culture velocity is maintained at 15–30 cm s^{-1} [50]. However, microalgae can grow quickly in bioreactor, photobioreactor with a high growth rate. Other than freshwater and nutrient media, the microalgae can grow in wastewater, marine water, or in sewage water. In the process of their growth, the microalgae also sequester carbon dioxide which makes the overall process a win–win situation where a feedstock for renewable fuel is derived and climate change is negated at least to some extent [49,52]. Several species and strains of microalgae have been utilized to produce oil and have shown potential to be utilized for the production of biodiesel.

Microalgae can also be grown by heterotrophic mode of cultivation, where carbon source is needed for its growth and subsequent lipid accumulation. Researchers have been investigating various carbon sources including that obtained from waste materials obtained from agriculture and food industries in addition to the conventional sources viz. glucose and fructose. The advantage of using algae as feedstocks is that the yield of the biodiesel can be increased from 10 to 100 times as compared to the conventional raw materials. However, microalgal species respond differently to environmental stress. Algal species that respond favorably to nutrient stress (e.g. *Chlorella vulgaris*) do so at the cost of a lowered growth rate [53]. Thus, the selection of the appropriate microalgae species is an important aspect in the production of high amounts of feedstock oil as fluctuations in the culturing conditions may lead to decrease in the biodiesel yield [54]. Some microalgal species also offer a high growth rate and a high lipid accumulation potential under nutrient stressed and optimal abiotic conditions. Table 2 depicts the microalgal feedstocks with oil content $\geq 50\%$ [55,56]. A high lipid content has been obtained from *Chlamydomonas pitschmannii* (51%), *Monoraphidium* sp. FXY-10 (56.8%). Cultivation, harvesting, and oil extraction poses challenges that have to be overcome for an economical production of microalgal oil for synthesis of biodiesel [57,58]. At present, the extensive processing steps involved in cultivation, harvesting, and oil extraction from microalgae for the synthesis of biodiesel are cost-intensive [59]. Microalgal oil comprises of constituents other than triglycerides and free fatty acids in substantial amounts. These constituents include hydrocarbons, sterols, waxes, sterol esters, free alcohols, pigments, phospholipids and glycolipids which are difficult to convert to FAAE. In addition, the algal lipid constituent varies according to the processing methods used in algae culture. Beal et al. [26] found that the triglyceride content decreased whereas, the other constituents (diglycerides, and free fatty acids) increased during processing of the algal oil. FFA was formed as a result of the breakdown of constituents such as phospholipids which were produced in the extraction process after the removal of lipid phase. The breakdown has been reported to be have occurred either physically or biochemically. While the physical damage has been attributed to processing methods (e.g. electro-mechanical pulsing), the biochemical damage has been attributed to enzymatic lipase activity [26]. Phospholipids and glycolipids present in lipid have been found to act as surfactant and are a major hindrance in deriving biodiesel of high quality [53].

The species of microalgae are numerous with varying lipid accumulating capacity under nutrient limited stressed conditions. Contrary to microalgae, plant species do not need stressed conditions for optimal oil productivity and the oil content varies to comparatively lesser extent. Plant species are also numerous though; not all are suitable to be diverted for the synthesis of biodiesel. Azam et al. [61] studied the prospects of 75 plants species and found 26 of them to be suitable as feedstocks for the synthesis of biodiesel on the basis of empirical values of saponification number, iodine value, and cetane number of the FAAE of the oil. Around, 300 feedstocks have been identified for the synthesis of biodiesel which includes edible, non-edible, used cooking oils, and fats [62]. Plant feedstocks along with those derived from waste animal fats and microalgae can amount to enormous feedstocks available for synthesis of biodiesel. However, the feasibility of the individual feedstocks depends on their oil constituents and the adherence of the fuel properties to the biodiesel specifications. High oxidation stability with a low cold filter plugging point is preferred for a biodiesel fuel. However an inverse relationship between a high oxidative stability (imparted with high saturated ester constituent) and better cold flow properties (imparted with high unsaturated constituents) of the biodiesel fuel [15]. Feedstocks with poor cold filter plugging points but may not be suitable for locations where cold climatic conditions persist. ASTM specification for biodiesel states the limit for linolenic acid which is 12% and the fatty acid containing four double bonds should not be more than 1% of the total constituent [61]. A high content of oleic acid (C18:1) offers a balance between the fuel properties (oxidation stability and cold filter plugging point) of biodiesel and its high

Table 2
Microalgal strains with oil content $\geq 50\%$.

Microalgae	Source	Lipid content (%)	Lipid productivity (%)	Reference
Nannochloropsis oculata	Cultivated in 1 L airlift PBR	50	3.6×10^{-3} $kg_{TG\text{-}FA}$ m^{-2} d^{-1}	[54]
Chlamydomonas pitschmannii	Freshwater lake	51	0.54 ± 0.9 g L^{-1}	[55]
Monoraphidium sp. FXY-10	Freshwater lake	56.80	148.74 mg L^{-1} d^{-1}	[56]
Chlorella vulgaris	Cultivated in sterilized medium	53	77.1 mg L^{-1} d^{-1}	[57]

PBR = Photobioreactor.
$_{TG\text{-}FA}$ = Pure triacylglycerol.

content in the microalgal or plant oils are preferable [63]. The presence of saturated fatty acids offers stability to the biodiesel and monounsaturated fatty acids allows for better low temperature properties with lesser compromise on the stability of biodiesel. Hence, the presence of saturated and monounsaturated fatty acids in a feedstock is desirable to obtain a better quality of biodiesel. The corrosion aspects of biodiesel also depend on the feedstocks used for production of biodiesel and a high concentration of unsaturated fatty acids can lead to greater oxidation rate caused by the fuel [64]. Another constituent that depends on the feedstock is acid value (AV). A high AV will limit the feasibility of the feedstock for alkaline transesterification of the feedstock. The alternative process for synthesis of biodiesel possessing a high acid value will be acid esterification which usually takes a longer time. Acid esterification followed by alkaline transesterification may also be adopted [15,65].

2.1. Lipid content and characteristics of microalgae and plant oil

The lipid content in plant and microalgal oils is of particular interest for their utilization in synthesis of biodiesel. The lipid constituent plays a vital role in the properties of biodiesel. The major constituent present in the plant oil is triacylglycerols (commonly called triglycerides) that is synthesized by esterification of glycerol with fatty acids in the presence of enzymes as catalyst. The plant oil is the major source of triglyceride in human diet [66].

Plant oils comprise of both saturated and unsaturated fatty acids. The major constituents in the non-edible oils are similar to the edible oils. Soybean oil which is the most common edible oil and constitutes 60% of world′s vegetable oil market is composed of five fatty acids: palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. The fatty acid profile of Sunflower oil (from the Brand Famila) also showed these five fatty acids as major constituents and other fatty acids in minor amount. Other edible oils (rapeseed, safflower) also have a similar constituent of fatty acids with minor differences in their composition. The fatty acid constituent in *Millettia pinnata* oil was similar to that of the edible oil (e.g. soybean and sunflower). The main difference observed in the composition of non-edible oils *M. pinnata* and *Madhuca longifolia* with edible oils is the presence of a monounsaturated fatty acid (i.e. oleic acid) as a major constituent (65.8 and 46.3%) in the former in comparison to 24.5 and 34.3% in soybean and sunflower oil, respectively. The linoleic acid composition was much less (12.1 and 17.9%) in *M. pinnata* and *M. longifolia* as compared to 53.0 and 51.17% in soybean and sunflower oil, respectively. Table 3 depicts the fatty acid composition of edible and non-edible plant oils [67–70]. However, apart from the triglycerides, the non-edible oils also contains significant amount of free fatty acids [15]. The non-edible oils also comprise of toxic constituents which limits their application. One of the widely used feedstock of synthesis of biodiesel, *J. curcas* contains phorbol esters. Hence, though *J. curcas* has protein content similar to soybean oil, it cannot be consumed for edible purpose and finds application in soil amendment and as feedstock for biofuel [71,72]. Similarly, *M. pinnata* (erstwhile *P. pinnata*) possesses furanoflavones, furanoflavonols, chromenoflavones, flavones and furanodiketones apart from triglycerides that makes the oil non-edible [73]. The toxic constituent in *M. longifolia* (erstwhile *M. longifolia*) is bitter saponins. *S. triguga* oil recently utilized for synthesis of biodiesel contains hydrogen cyanide in the concentration of 0.03–0.05% which hinders in either its saponification or transesterification. To make the oil saponifiable, the cyano-glucosides is hydrolyzed under pressure using zinc oxide and sodium bisulphate which is followed by settling and removal of aqueous layer [29]. Azam et al. [61] studied the lipid profile of 75 plant species found in India comprising lipid content more than 30% in their seed or kernel. The saponification number of the plants varied from 169.2 to 312.5. Whereas, the iodine value, which the measures the level of unsaturation varied from 4.8 to 212. Based on the lipid profile of the plants, its SV, IV, and cetane number (CN), Azam et al. [61]repotted 26 plants to be suitable for synthesis of biodiesel among the selected plant species. These species possessed SV ranging from 193 to 205.2; IV ranging from 49.9 to 98.4; CN ranging from 51.09 to 61.55. The minimum value of CN possessed by biodiesel has been specified by USA (ASTM D 6751), Germany (DIN 15606), and European Standard (EN 14214) to be 47, 49, and 51, respectively. A high composition of saturated fatty acids in oil will give a high value of CN. IV has a profound effect on the fuel properties of biodiesel as presence of unsaturated components prevents biodiesel against solidification. However, a large degree of unsaturation leads to polymerization of biodiesel upon reaction with atmospheric air. A maximum limit of 115 has been specified by EN 14214 for IV. Also, there exists an inverse relation between CN and IV. Though, a high value of CN is desired in a fuel, a very high value will lead to solidification of biodiesel and hence an upper limit of 65 for CN has been specified in US biodiesel specification Azam et al. [61].

The lipid constituents of plant and microalgal oils are quite different. The lipid content in plant oil that has shown potential and has been utilized for synthesis of biodiesel varies from 33% (*P. pinnata*) to as high as 73%. (*Canarium commune*). However, the commonly used plant feedstocks (*J. curcas*, *M. indica*, and *Schleichera oleosa*) have oil content of 40% [15]. The composition of triglycerides present in the plant based feedstock oils varies from saturated and monounsaturated to polyunsaturated fatty acids. However, the microalgal oil, in addition to triglycerides may also contain substances such as hydrocarbon, alcohol, and wax which cannot undergo either esterification or transesterification and hence have to be separated or otherwise they will remain in the final product.

Microalgal oils may also contain alkane like hydrocarbons which have a high calorific value and low viscosity. Microalgae are known to produce neutral lipids comprising mainly of triacylglycerols when they are subjected to environmental stress. Complete nutrient stress results in a higher accumulation of neutral lipids as compared to selective nutrient stress [74]. The synthesis of neutral lipids has been suggested to be protective mechanism for the cells to adapt under stressful conditions [75]. The lipid constituent in the microalgal oil is made up of saponifiable as well as non-saponifiable matter. The neutral lipids comprises of the saponifiable matter viz. triglycerides and free fatty acids. The total lipid constitutes of neutral lipids, pigments, phospholipids and glycolipids [48]. Despite neutral and polar

Table 3
Fatty acid composition edible and non-edible of plant oils.

Plant oil	C12:0 (%)	C16:0 (%)	C16:1 (%)	C17:0 (%)	C17:1 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C20:0 (%)	C20:1 (%)	C22:0 (%)	C22:1 (%)	C24:0 (%)	Reference
Soybean oil	–	11.5	–	–	–	4	24.5	53	7	–	–	–	–	–	[67]
Sunflower oil	0.53	6.14	0.09	0.09	0.06	4.11	34.2	51.17	2.23	0.17	0.17	0.41	0.53	–	[68]
Millettia pinnata	–	8.9	–	–	–	8.2	65.8	12.1	–	0.9	0.9	2.8	–	0.4	[69]
Madhuca longifolia	–	17.8	–	–	–	14	46.3	17.9	–	–	–	–	–	–	[70]

lipids being able to be converted to biodiesel, neutral lipids are considered to be the best substrate for production of FAAE as they can be easily transesterified [76]. The focus in recent times has been a high lipid accumulation in the cells of microalgae species. Various studies have been done by researchers to explore the species with a high oil/lipid accumulating potential. However, the lipid content varies significantly among the microalgae species. The theoretical values of microalgal oil that can be extracted from microalgae have been reported to be high. However, not all species have shown a high amount for the fatty acid accumulation and biomass productivity. The oil content is found to be highest in *Botryococcus braunii* (25 to 75%), but its productivity is poor. The commonly grown microalgae (species of *Chlorella*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nanno-chloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum* and *Porphyridium*) have oil content in the range 20–50% [77]. Abou-Shanab et al. [78] isolated 45 microalgae from a freshwater lake and on the basis of their morphology studied the lipid accumulating potential of five species amongst them. The lipid content potential varied significantly for the selected species and possessed a comparatively low content of 26% (of dry weight) for *C. vulgaris* and a high content of 51% (of dry weight) for *C. pitschmannii*. Though, a considerable yield of total lipid was observed from the three microalgae (*C. vulgaris*, *Neochloris oleoabundans*, and *Cylindrotheca closterium*) taking in account the dry weight of the biomass (viz. ranging from 17 to 23%), the lipid content comprising of triacylglycerols was less and varied from 9 to 14% of dry weight for the three microalgae with the highest value in the range 11–14% of dry weight was obtained in case of *C. vulgaris* [27]. Some species have shown a high triglyceride accumulation efficient in their lipid profile. *Scenedesmus obliquus* and *Chlorella zofingiensis* microalgal species have shown accumulation of 35% triglycerides of their dry weight. A high triglyceride productivity of 322 and 243 mg L^{-1} d^{-1} has been observed [28]. Kaiwan-arporn et al. [79] reported that the cyanobacteria, *Synechocystis aquatilis* comprised mainly of polyunsaturated fatty acids (primarily linoleic acid and α-linolenic acid). Chinnasamy et al. [80] demonstrated that only about 63.9% of the algal oil extracted could be converted to biodiesel. The final recovery of the FAAE further reduced to 38.7% due to loss during base transesterification and purification of the product. There exists a trade-off between the productivity of microalgae and its lipid accumulating capacity [21]. Thus, when environmental conditions are controlled for high lipid accumulation, the microalgae divert energy to form lipids thus compromising with its productivity. Similarly, high biomass productivity in microalgae is achieved when not subjected to stress for high lipid accumulation [21]. Hence, a balance between the lipid production and biomass growth in microalgae has to be maintained for on optimum production of oil. The chain length of the fatty acid present in the feedstock influences the viscosity of the biodiesel. A longer chain length of the fatty acid causes a high viscosity [81]. Presence of high content of saturated fatty acid also contributes to a high viscosity. Winterization can be done to reduce the content of saturated fatty acids from oil. The technique can be used to ozonize the oil which results in a smaller size of crystals formed at low temperature. Alternatively, biodiesel can be cooled and the crystals can be removed by filtration [9]. Kerschbaum et al. [9] have proposed winterization of oil/biodiesel from waste cooking oil by using micro heat exchangers which resulted in reduction of saturated fatty acid methyl esters from 21.3 to 9.6% which led to lowering of the cold filter plugging point by 11 °C.

Another limiting factor for the usage of microalgal oil as feedstock for synthesis of biodiesel is the presence of phospholipids which interfere with lipase (used to catalyze the reaction) and substrate [58]. A few microalgal species (*Chaetoceros gracilis* and *Tetraselmis tetrathele*) have been found to grow in saline water and produce high levels of lipids. These microalgae respond to sodium hydroxide thus could be harvested using flocculation. *B. braunii* has been found to grow in saline waters, contains a high level of hydrocarbons (up to 80% of the dry biomass) [82]. The method utilized a silica cartridge for the fractionation of the total lipids to neutral lipids, glycolipids, and phospholipids. Different solvent mixtures with varying ratios were used for their elution. After conditioning the adsorbent with chloroform, the neutral lipid, glycolipid, and phospholipid were eluted from the adsorbent bed with chloroform: acetic acid (9:1, v/v) mixture, acetone: methanol (9:1, v/v) mixture, and methanol, respectively. The fractions were then collected in conical vials and evaporated to dryness under nitrogen [20]. The same method was adopted by Damiani et al. [83] for the fractionation of lipid (neutral, glycolipid, and phospholipid) of *H. pluvialis.* Voorenet al. [54] has reported high oil content from *Nannochloropsis oculata* amounting to 50 and 43% (dry weight) of fatty acid and pure triacylglycerol, respectively. A high lipid productivity of 3.6×10^{-3} kg of triacylglycerol m^{-2} d^{-1} has been reported. Voorenet al. [54] reported that the nitrogen starvation to enhance the lipid productivity results in simultaneous decrease in growth kinetics. Therefore, in addition to nitrogen starvation, the amount of light received per cell should be high for high triglyceride accumulation in microalgal oil. The light received per cell is dependent on the incident photons flux density, biomass optical properties and concentration, geometry of photobioreactor (depth of the culture being the prominent).

Marine microalgae provide an opportunity for low cost microalgae cultivation in the natural environment. Doan et al. [76] studied 96 strains of marine microalgae (isolated from the coastal waters of Singapore) that possessed a high biomass productivity and intracellular lipid content for utilization as feedstock for biodiesel. Among these 96 microalgal strains, 26 were characterized on the basis of high growth rate ($0.41-0.95$ d^{-1}), biomass concentration ($0.04 \pm 0.01-0.39 \pm 0.09$ g L^{-1}), and lipid content ($0.9 \pm 0.1-44.8 \pm 1.7$% g^{-1} DCW) lipid content. Among the species, *Skeletonema costatum*, *Chaetoceros* sp., and *Thalassiosira* sp. showed comparatively high growth rate of 0.95, 0.87, and $0.61-0.85$ d^{-1}. However, in terms of lipid yield, strains of *Nannochloropsis* sp. were found to be highest with the lipid content up to 45% g^{-1} DCW. Owing to their high biomass productivity and high intracellular lipid content, they have been projected to be a prominent feedstock for biodiesel production. However, when the transesterification of the oil derived for *Nannochloropsis* species were carried out, the yield reported (25–51%) was much lower than that specified by the EN norms (96.5%) [76].

Vijayaraghavan and Hemanathan [84]reported the production of biodiesel from freshwater microalgal oil. The lipid content of the microalgae was reported to be 45 ± 4%. Knothe and Kenar [85], based on the physico-chemical properties debated that the products obtained from microalgal species utilized by Vijayaraghavan and Hemanathan [84]were hydrocarbons. The density of the product obtained by Vijayaraghavan and Hemanathan [84]was 0.801 kg m^{-3}, whereas the density of biodiesel is in the range of 0.86–0.89 kg m^{-3}. The flash point of the biodiesel was also low (98 °C) as compared to the minimum 130 °C specified for biodiesel. This necessitates the proper identification of the alkyl esters and other constituents in biodiesel derived from microalgal oil. The choice of feedstock is governed by the available factors that include the agriculture, sociology, geopolitics, and economy of a nation. Table 4 depicts the lipid profiles of some of the microalgal species. It is observed that the lipid constituent is dominated by unsaturated fatty acids [63,80,86–87].

2.2. Lipid extraction from microalgae and plant

Plant or microalgal oil after selection can be used for the synthesis of either of the bio-fuel, i.e. biodiesel, bio-oil or biochar [88]. Of these products, biodiesel offers a high calorific value and is preferred for

Table 4
Fatty acid profile of some microalgal strains studied for biodiesel production.

Microalgae	C14:0 (%)	C15:1 (%)	C16:0 (%)	C16:1 (%)	C17:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:2n6 (%) trans	C18:2n6 cis	C18:3 (%)	Reference (%)
Botryococcus sp.	–	–	4.8	–	0.8	4.3	55.7	34.2	–	–	0.2	[63]
C. vulgaris	–	–	ND	–	0.1	3.4	16.3	79.4	–	–	0.1	[63]
Scenedesmus sp.	–	–	–	–	0.7	3.0	57.2	36.8	–	–	2.2	[63]
Refined algal oil (from consortium of 15 native algae of USA)	1.4	5.9	17.6	–	–	–	14.9	–	9.6	11.8	38.3	[80]
Chlorococcum sp.	–	–	~19	~4	–	~3	~63	~4	–	–	–	[86]
Neochloris oleoabundans	–	–	23–30	–	–	–	30–43	18–23	–	–	5–12	[87]

ND: Not detected.

synthesis. However, based on the feasibility and requirement of the end products, either of these products may be synthesized. While, esterification and transesterification are utilized for synthesis of biodiesel, pyrolysis is the process adopted for the production of bio-oil or biochar [49]. For the production of either of these utility products, the process for extraction of oil is lengthy which starts with choosing an ideal feedstock that could be cultivated and harvested with a high biomass (dry) productivity and possessing a high oil/lipid (of triglycerides and free fatty acids) content.

Plant oils that are derived from either seed or kernels constitute a known percentage of oil varying from 30 to 35% [61]. Extraction of the oils from plants is simple, usually by a mechanical expeller followed by soxhlet extraction method using a solvent. Extraction of microalgal oil involves numerous lengthy processes. The steps involved in harvesting and expulsion of microalgal oil can include sedimentation, centrifugation, filtration, ultra-filtration, additional flocculation to remove algae from water, drying, and solvent extraction [2]. Drying of biomass and extraction of oil from the cultured microalgae could account for 80% of the total energy consumption [50]. The harvesting can be done by several techniques namely centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, or through electrophoresis. The mode of dewatering differs among the microalgal species and depends upon the microalgal properties like cell size, culture density. Centrifugation, though found effective bares a high operating cost. Flocculation, gravity sedimentation has been found to concentrate microalgae culture. Hence, a combination of centrifugation with other techniques (flotation or gravity sedimentation) for an efficient dewatering may prove more economical. Filtration of microalgae utilizes membranes through which the microalgae suspension is passed. The challenge with the technique is that specific pore size of the screens should be tailor-made for the specific species of microalgae. High concentrations of microalgae cause blockages of the screen, whereas, low concentrations of microalgae result in inefficient capture. Gravity sedimentation offers a cheap technique whereby natural sedimentation of the microalgae occurs through lamella separators and sedimentation tanks. The orientation of plates in lamella separators offer increased settling areas [30]. After harvesting, biomass is dried using various methods like solar drying, oven drying and freeze drying. Of these techniques although solar drying is the most time consuming but it is most energy efficient technique. Dried biomass is subjected to solvent extraction. Disruption of cell wall using microwave, sonication and osmotic shock facilitates the lipid extraction.

3. Conversion of microalgae and plant oil to biodiesel

3.1. Catalysts for esterification/transesterification

3.1.1. Chemical catalysts for esterification/transesterification

The catalyst plays an important role in the esterification or transesterification of lipid/oil obtained from plants and microalgae. Catalysts that have been used for esterification and transesterification of plant oils include heterogeneous and homogeneous chemical catalysts [89–92]. The mechanism of esterification/transesterification from the homogeneous and heterogeneous catalysts are similar. Homogeneous base catalysis for transesterification is a three step process which starts with the formation of alkoxide from the alcohol. The nucleophilic alkoxide attacks the carbonyl carbon group in the triglyceride to form a tetrahedral intermediate. The intermediate reacts with an alcohol to regenerate the alkoxide in the second step. The rearrangement of the intermediates leads to formation of diglycerides and FAAE. In the last two steps, two molecules of FAAE are formed when diglyceride and monoglyceride are converted to monoglyceride and glycerol, respectively. In acid catalysis, the carbonyl group of the triglyceride gets protonated which leads to carbocation. The protonated carbon is attacked by an alcohol that results in formation of an intermediate. In the next step the intermediate eliminates glycerol that results in formation of an ester while the catalyst is regenerated [17,93]. In the heterogeneous Lewis basic catalyzed reaction (CaO and MgO) formation of the alkoxide group is pertinent. In general, the Brönsted acid group of catalysts is found to be active mainly in esterification reactions and Lewis acid catalysts are more active in transesterification reactions [92]. A high biodiesel yield with feedstocks of acid value <4.0 mg KOH g^{-1} amount to almost complete conversion (100%) has been reported by the researchers using homogeneous base catalysts. The biodiesel yields with heterogeneous base catalysts are also high amounting to $>98\%$, although the reaction rate is comparatively slow compared to those catalyzed with homogeneous catalysts. The reason is due to three phase system in the heterogeneous catalyzed reaction which causes a mass transfer limitation during reaction. In the heterogeneous catalyzed reaction, the adsorption of reactants (oil and alcohol) and desorption of products {FAAE (biodiesel), di-, mono-glyceride, and glycerol} should occur on the surface of the solid (acid or base) catalyst at a considerable rate [93]. The advantage using the heterogeneous catalyst is its easy separation from the products and production of glycerol of high purity (98%) as compared to that with homogeneous derived catalyst (80%) [42].

Though, homogeneous catalysts are few, there is a vast range of the solid (heterogeneous) catalysts that have been explored to catalyze the transesterification reaction. Common homogeneous base catalysts used in transesterification include sodium hydroxide, potassium hydroxide, and sodium methoxide. Sulphuric acid is utilized as homogeneous acid catalyst for esterification. The application in the homogeneous acid and base catalysts in biofuel industry have numerous disadvantages e.g. corrosion of the reactors, storage tanks, and engines [94]. In addition, their removal from the biofuels through aqueous quenching results in the formation of stable emulsions and soap and are energy intensive [2]. The heterogeneous catalysts utilized in synthesis of biodiesel are grouped as solid acid and solid base catalyst. Solid acid catalyst includes a wide range of chemicals viz. resins, tungstated and sulfated zirconia, polyaniline sulphate, heteropolyacid, metal complexes, sulfated tin

oxide, zeolite, acidic ionic liquids, and others have been used by researchers [90]. Solid base catalysts also include a wide range of catalysts viz. calcium oxide, hydrotalcite (also called layered double hydroxide), alumina and zeolites [89]. One of the major issues with the use of homogeneous and heterogenous catalysts is its removal from the product. Removal of leached constituents of heterogeneous catalysts is a major issue in synthesis of biodiesel. While a large number of heterogeneous catalysts have been utilized for the synthesis of biodiesel from the plant based oil, the activity of several these catalysts on the microalgal based oil is yet to be tested [90].

Conventional catalysts were designed on the basis of feedstocks that were refined and constituted of minimal impurities. In recent times, the non-food based feedstocks have been extensively used in the production of biodiesel which pose new challenges for a high catalytic activity for esterification/transesterification. These challenges include water tolerance, pore size, and dimensionality of the channel system for the solid catalyst for its application in the esterification/transesterification reaction [2]. The plant oil has shown a high yield and conversion when catalyzed with homogeneous catalyst in a short time (around 1 h). With heterogeneous catalysts a high yield and conversion of plant derived oil to biodiesel has been obtained, though with comparatively longer time.

3.1.2. Biocatalyst for esterification/transesterification

Enzyme lipases are employed as catalyst for biodiesel synthesis. Enzyme catalysts can be effectively used for low quality feedstock with high free fatty acid content. Lipases catalyze both esterification as well as transesterification. Quality and yield of product is high. Enzymes have shown a good catalytic efficiency in the conversion of oil to biodiesel with purity ranging from 90 to 96%. A high purity has been attributed to the “polishing” cycles where the contaminant(s) also get converted to biodiesel. However, the enzymatic mode of catalysts suffers from constraints. Besides it being costly, water is usually required to enhance the activity of enzyme that in turn causes accumulation of fatty acids in biodiesel. This requires “polishing” steps by additional treatment of the remaining fatty acids with the enzyme. If fatty acid concentration is $>1\%$, the shift in reaction equilibrium requires a high alcohol concentration, thus causing irreversible damage to the enzyme [95].

There is immense scope for usage of enzymes as catalyst in the synthesis of biodiesel from plant or microalgae derived oil. The enzymes have constrains in the current scenario in its application as catalyst for synthesis of biodiesel. However, there are possibilities of improvement in the process where enzymatic methods can be adopted at relatively low temperatures and atmospheric pressure which will lead to reduction in consumption of energy. Enzymes pose a high selectivity and specification and can lead to a high conversion of oil to biodiesel resulting in a high quality product. When an enzyme (particularly lipase) is being used as a catalyst, methanol has to be added in stepwise to avoid the lipase deactivation. In the process, one molar equivalent of methanol with respect to the oil is added at a time and the process is repeated thrice. There have been limited studies on the conversion of microalgal oil to biodiesel, either chemically or enzymatically [96]. Hama and Kondo [57] states that the present price of enzymes is higher than its alkali counterpart. The enzymes are also prone to inactivation which may limit its applicability to industrial scale production [97].

To tackle these constraints researchers are looking for possible solutions. Use of immobilized enzymes provides advantage of its easy separation and reusability. Immobilization also provides stability to the enzyme. The advantage with the process is that the lipase (*Candida antarctica*) can be reused for more than 50 cycles. Immobilized enzyme can be used in continuous reactors which are easy to scale up to industrial scale. Lipases from different sources has varying specificity and stability thus new approaches to using lipase mixtures are being employed to achieve high yields and better performance. A whole cell catalyst comprising of two lipases {*C. antarctica* B (Novozyme 435) and *Thermomyces lanuginosus* (Lipozyme)} were co-expressed on the surface of *Pichia pastoris* cell through self-immobilization that avoids the procedures of complex immobilization. A high biodiesel conversion (ca. 95.4%) was observed in the first run of the experiment at a moderate temperature (40 °C) in 12 h at the stirring rate of 200 rpm. However, the activity of the catalyst decreased to 90% in the second run and further in the subsequent runs. Hence, for the ability to reuse the catalysts further improvements are required [97]. In future with advances in enzyme catalysts for biodiesel production, it has potential to provide greener and sustainable conversion strategy for biodiesel production. Tables 5 and 6 describe various catalysts used for the conversion of different plant and microalgal oil, respectively [25,93,98–111]

3.2. Reaction kinetics in the synthesis of biodiesel

The kinetics is an important parameter to be studied in the production of biodiesel as it depicts the activation energy of the reaction and the time involved for the completion of the reaction. The esterification/transesterification of the fatty acids present in the oil/fat reacts at a moderate rate. Based on the rate constants, the conversion of triglyceride to diglyceride has been found to be the slowest reaction in transesterification [112]. In the presence of excess alcohol, the kinetics of the transesterification is pseudo-first order for forward reaction and second order for the backward reaction [17].

As the reaction is mass transfer controlled due to the low miscibility of the two reactants, a subsequent low initial reaction rate is observed. However, the mass transfer limitation is overcome by mechanical means of agitation. The formation of methyl esters results in the formation of single phase of reactants by acting as a mutual solvent and increased the equilibrium concentration of triglycerides in methanol. The production of methyl esters increases the overall rate of mass transfer of the reactants. Hence, the production of methyl esters increases the solubility of triglycerides in methanol as well as mass transfer coefficient. It has been found that the intrinsic reaction rate during the transesterification reaction is not fast enough to consume all the triglycerides as they react with alcohol thus accumulating the reactants in the reaction vessel taking considerable time for reaction to complete. The catalyst concentration directly affect the reaction rate, whereas, the agitation speed directly affects the mass transfer rate. Agitation enhances the contact between the two phases of the reactants thus increasing the surface area. Thus a high concentration of triglycerides in the methanol phase is an indication high reaction rate. Csernica et al. [113] states that the transesterification reaction gets accelerated under mass transfer control as well as reaction control. It is predicted that the acceleration in the transesterification reaction is not necessarily caused by the change in the rate limiting step but from the phase transition from two phases to a single phase. The consumption of antioxidants in FFA based biodiesel has been found to follow the first order reaction kinetics [114]. During the overall reaction, the first step that involves the conversion of triglycerides to diglycerides was observed to be the rate determining step in the production of biodiesel. The reaction rate constant for the first step of conversion (triglyceride to diglycerides) was 0.0311 which was significantly lower than the second (diglycerides to monoglyceride) and third step (monoglycerides to triacetylglycerol) of reaction with values of 0.1124 and 0.1129, respectively [115].

Table 5
Conversion of various plant oils to biodiesel.

Feedstock (oil)	Catalyst	Reaction conditions				Biodiesel yield (*Y*)/ conversion (*C*) %	Reference
		Catalyst loading % wt/oil wt	Molar ratio Alcohol: oil	Temperature (°C)	Time (h), stirring (rpm)		
Corn	p-toluenesulfonic acid	4	10:1	80	120	$Y=97.1$	[98]
Soybean	Strontium oxide (SrO)	3	12:1	65	30	$Y=95$	[99]
Soybean	Calcium oxide (CaO)	8	12:1	65	90	$Y=95$	[100]
Soybean	Nd_2O_3 loaded with KOH	6	14:1	60	1.5,–	$Y=92.41$	[101]
Mixed feedstock (soybean and rapeseed)	NaOH	0.8	5:1	55	2, 1600	$Y=94$	[102]
Sunflower	NaOH	1	6:1	60	2, 600	$Y=97.1$	[103]
Jatropha curcas	Li-CaO $Fe_2(SO_4)_3$	5	6:1	60	3, 300	$Y=100$	[93]
J. curcas	lipase (*Enterobacter aerogenes*)	50 U g^{-1} [a]	4:1	55	48, 200	$Y=94$	[25]

[a] Lipase in activity in unit/g.

Table 6
Conversion of various microalgal oils to biodiesel.

Microalgae	Catalyst	Reaction conditions				Biodiesel yield (*Y*)/ conversion (*C*) %	Reference
		Catalyst loading (% wt/oil wt)	Molar ratio Alcohol: oil	Temperature (°C)	Time (h), stirring (rpm)		
Chlorella protothecoides	Sulfuric acid	100	56:1	30	4, 160	$Y=60$	[104]
Oedogonium sp.	Sodium hydroxide	–	–	–	3, 300	$Y>90$	[105]
Spirogyra sp.	Sodium hydroxide	–	–	–	3, 300	$Y>90$	
N. oculata	Al_2O_3 supported CaO	2	30:1	50	4, 1100	$Y=97.5$	[106]
Chlorella protothecoides	*Candida* sp. 99–125 sp. lipase	30	3:1	38	12, 180	$C=98.15$	[107]
C. vulgaris ESP-31 Extracted oil	*Burkholderia* lipase	1203.11 U g^{-1} [a]	12.35:1	40	48, 600	$C=72.12$	[108]
C. vulgaris ESP-31 Wet microalgal biomass	*Burkholderia* lipase	1203.11 U g^{-1} [a]	67.93:1	40	48, 600	$C=97.25$	[108]
Dinoflagellate	Esterification—sulfuric acid	1	30:100 (v:w)	65	2	$C=100$	[109]
	Transesterification—potassium hydroxide	2	12:1	65	0.5, 100		
Chlorella pyrenoidosa	*Penicillium expansum* lipase (PEL)	20	3:1	50	48	$Y=90.7$	[110]
Microcystis aeruginosa NPCD-1	*Candida antarctica* lipase (Novozyme 435)	10	12:1	50	48, 150	$C=67.84$ $Y=98.10$	[111]

[a] Lipase in activity in unit/g.

In general, the determination of kinetics requires collection of numerous samples. This has been done by gas chromatography and high performance liquid chromatography. However, these processes are relatively lengthy as they require the derivatization of the sample. Hence, alternative methods have been adopted by researchers for fast determination of the reaction kinetics. Birla et al. [116] used the nuclear magnetic resonance as a rapid method in the characterization of fatty acid alkyl ester content in biodiesel. The first order kinetics of the reaction was established with activation energy of 79 kJ mol^{-1} and frequency factor of 2.98×10^{10} min^{-1} utilizing waste frying oil and snail shell derived CaO as catalyst. Vujicic et al. [117] reported the transesterification is actually a fourth order reaction with the assumption that three moles of alcohol reacts initially with one mole of triglyceride. But, as an excess amount of alcohol is usually employed in the transesterification, the reaction is assumed to be follow pseudo first order kinetics. In the transesterification of sunflower oil using CaO as heterogeneous catalyst, a negative influence of the reaction time on the yield of biodiesel was observed. A drop of activation energy from 162.1 to 101.0 kJ mol^{-1} was observed when the reaction time was increased from 1.5 to 5.5 h. The reaction rate constant was studied at various temperatures and found to be highest during 5th (21.74×10^{-3} min^{-1}) and 2nd h (109.67×10^{-3} min^{-1}) at 60 and 80 °C, respectively. The highest reaction rate of 175.40×10^{-3} and 220.86×10^{-3} min^{-1} was obtained at 1st h when run at 100 and 120 °C, respectively. The first-order kinetics was also proposed by Zhang et al. [118] for the transesterification of palm oil with dimethyl carbonate using KOH as homogeneous catalyst. A good linearity between ln k and $1/T$ was observed in the temperature range of 65, 70, and 75 °C which validated the pseudo first order hypothesis of the reaction. Santos et al. [119] calculated the activation energy (E_a) for the volatilization of sunflower oil and biodiesel derived from sunflower oil. The E_a was obtained as a function of the conversion for the volatilization process and was in the range of ca. 50–75 kJ mol^{-1} for biodiesel (derived from sunflower oil) and ca. 170–210 kJ mol^{-1} for the sunflower oil. Table 7 shows reaction kinetics in the production of biodiesel [116,117,120–122]. The high E_a for the thermal volatilization of sunflower oil has been advocated for its feasibility as a potential feedstock bearing a high stability. As the collection of numerous samples (conversion of oil to biodiesel) at subsequent time interval is needed for the study of kinetics and the conventional mode of analysis by gas chromatorgaphy, high performance liquid

Table 7
Kinetics studies in transesterification reaction.

Feedstock	Catalyst	Kinetics	Activation energy kJ/mol	Pre-exponential factor	Reference
Waste frying Oil	Calcined snail shell (CaO)	First-order	79	2.98×10^{10} min^{-1}	[116]
Sunflower	CaO	Pseudo first-order	101	–	[117]
Palm oil	KOH	Pseudo first-order	79.1	1.26×10^{9} min^{-1}	[120]
Soybean oil	$Ca(C_3H_7O_3)_2/CaCO_3$	42.096	19260	121	
Enteromorpha compressa (algal oil)	Lipase (Novozym 435)	First-order	73.15489	–	[122]

chromatography, and thin layer chromatography is time consuming. Fedosov et al. [95] utilized a dye, Nile Red for monitoring the progress of esterification reaction of oleic acid using fluorescent probe. Nile Red, being soluble in organic liquids changes its quantum yield (forming hydrogen bonding with organic molecule) in the presence of hydrophilic group and causes a red shift of its emission spectrum. In the esterification reaction, the substrates (oleic acid and organic alcohol) have hydrophilic group which on reaction forms two products (hydrophobic molecule and water). While, water gets separated from the oil phase, the hydrophobic molecule is 'neutral' towards fluorophore. Thus, there is disappearance of red shift emission spectrum with the progress of reaction.

3.3. Refining techniques for crude biodiesel

Biodiesel should be of high quality which is indicative through its FAAE content (w/w) and thus adherence to the specifications. The purity of the biodiesel is dependent on several factors viz. fatty acid composition of the feedstock, presence of constituents other than fatty acids in the oil, production process adopted for its synthesis and the refining methods adopted for the crude biodiesel [120]. The crude biodiesel contains impurities i.e. products other than fatty acid alkyl ester that include soap (formed due to saponification, if an alkali is used as a catalyst), tri-di-and mono-glyceride (due to un-complete reaction), alcohol (unreacted), and glycerol (un-removed during separation with FAAE). Some other constituents may also be present in minor quantity in the oil, if already present in the feedstock(viz. saponins in *M. indica* oil) [14]. These minor constituents may comprise of toxic components present in many non-edible feedstocks. The refining technologies for biodiesel derived from non-microalgal based feedstocks are well documented in literature. One of the most common techniques widely adopted include an easy method such as washing of the crude biodiesel (for removal of residual catalyst, glycerol, alcohol, and tri-, di-, mono-glycerides) and subsequent removal of water either by an anhydrous compound (viz. sodium sulfate) or through rotary evaporator [123]. Karaosmanoğlu et al. [124] reported washing of the crude biodiesel by hot distilled water (50 °C) to obtain biodiesel of high purity 99% (w/w) FAAE and a high refining yield (86.3%). The water washing steps may be curtailed to some extent by utilizing heterogeneous catalysts for the synthesis of biodiesel, where the solid catalyst can be separated by filtration and alcohol can be removed by rotary evaporator. An acid is usually used (ortho-phosphoric acid) to neutralize the alkali (used as catalyst in the transesterification) so that less water is needed for removal of the residual catalyst and emulsion formation is reduced. The alcohol is removed prior to wet washing to prevent its entrance in the wastewater and to allow reuse [125].

Despite the effectiveness of water washing for the removal of contaminants, it is an energy intensive process and causes loss of FAAE and whilst generating wastewater [123,125]. Researchers have explored methods to curtail the energy consumption while refining the crude biodiesel so that it fulfills the international specifications (ASTM D6751 and EN 14214). Atadashi et al. [123] discussed various refining technologies for the purification of crude biodiesel and reported membrane refining process to be less energy consuming and generating minimal wastewater. The technologies that have been described for refining of crude biodiesel are wet washing, dry washing, and through membrane refining. Wet washing of crude biodiesel by water was efficient in the removal of methanol and free glycerol and meeting EN 14214 specifications. The dry washing process utilized either magnesol or ion exchange resins were inefficient in the removal of methanol. Using the membrane technology, the removal of the constituents other than FAAE (triglycerides, residual glycerol) has been advocated as an efficient method for purification of biodiesel. The use of enzymatic catalysts simplifies the refining of the product, though it has the disadvantage of slower conversion of triglycerides to FAAE. The separation and purification of biodiesel has been found to be simpler with refined vegetable oil due to less content of fatty acids and water than in unrefined vegetable oils [120].

Though the separation process adopted for the removal of biodiesel from glycerol by washing with water is efficient, the equilibrium solubility of water in esters is higher than that specified for water in pure biodiesel. Hence, an additional step is incorporated that demands the removal of water from biodiesel by either vacuum driers or passing it through anhydrous sodium sulfate [126,127]. Another disadvantage of using water as a medium for purification of esters is the loss of ester content as it gets washed away with the waste water. The separation of glycerol from biodiesel is necessary for the biodiesel fuel to comply with the international specifications that restricts the maximum limit to 0.02%. Gomes et al. [126] used microfiltration with ceramic membrane to separate biodiesel from glycerol. The ceramic membranes with pore diameter of 0.2, 0.4, and 0.8 μm were found to efficiently separate biodiesel from glycerol. When transmembrane pressure of 2.0 bar was applied with a 0.2 μm membrane, the glycerol was found to remain on the membrane with an efficiency of 99.4%. Ren et al. [127] demonstrated a fixed bed reactor packed with anion exchange resin as a heterogeneous catalyst in a continuous process for the synthesis of biodiesel and found complete separation of glycerol due to its adsorption on the resin. When microalgae are used as feedstock, constituents other than triglycerides and free fatty acids are also present whose removal may or may not be necessary. The biodiesel is usually washed till the pH is neutral. Falahati and Tremblay [12] utilized various feedstocks (canola, sunflower, and corn) for the synthesis of biodiesel. The water and FAAE ratio of 1:4 was adopted for washing of crude biodiesel from edible oil only once which was followed by either dry washing or cold filtration to remove monoglycerides. The diglycerides and triglycerides are not removed by water washing. Glycerol concentration after the water washing of FAAE was within the ASTM D6751 and EN 14214 specifications. It was found that the biodiesel synthesized by membrane reactor required less water washing due to particulate free nature of the permeate.

4. Properties of biodiesel synthesized from microalgae and plant oil

4.1. Effect of feedstocks on fuel properties and exhaust emissions in a CI engine

It is widely accepted that the long term exposure to particulate matter causes adverse health effects leading to lung cancer and

cardiopulmonary mortality [128]. Hence, the reduction in the exhaust emissions from the combustion of biodiesel is an important aspect for the advocacy of its usage in CI engines. The fatty acids present in plant oils differ from that of microalgal oils. While, the plant oil comprises of a mixture of unsaturated and saturated fatty acids, the microalgae comprises mainly of unsaturated fatty acids that comprise of four or more double bonds [129]. The presence of high a constituent of polyunsaturated fatty acids in the feedstock can lead to a lower stability of biodiesel [130]. Linolenic acid that contains three double bonds should not be present in excess of 12% and the double bond should not be excess of 1% as per the specification for biodiesel. A high level of unsaturation for biodiesel will result in oxidation of the fuel and will render it off-specification. The fatty acid chains in the lipids present in microalgae can range from C11 to C26, though the common chain length usually observed is C14 to C22.The fatty acid constituent may be saturated or unsaturated (with up to six double bonds) [82]. In early work carried out by Azam et al., [61] the fatty acid profiles of 75 plant species with oil content $>30\%$ were examined. Based on the fatty acid content, only 26 species showed potential for their application as feedstocks for the synthesis of biodiesel. The parameters that were taken in consideration were saponification value, iodine value, and cetane number that were determined empirically. The fatty acid composition of the feedstock along with FFA content, moisture, impurities, and unsaponifiable constituents have a profound effect on some of the physico-chemical properties of biodiesel. These include density, acid value, viscosity, flash point, cetane number, oxidation stability and cold flow properties. The cetane number of the biodiesel increases with increasing chain length of the fatty acids and increasing saturation. The presence of high content of unsaturated components (C18:2 and C18:3) results in a low cetane number of biodiesel. The cold filter plugging point (CFPP) indicates the cold flow property of biodiesel has a lower value for the feedstock comprising of unsaturated fatty acids. A low CFPP is preferable as the fuel (biodiesel) will gets solidified at a lower temperature [129]. Tang et al. [131] studied the biodiesel obtained from soybean, cottonseed and poultry fat stored at 4 °C and observed that the precipitates were formed, the extent of which depended on the feedstock and blend concentration. The cottonseed feedstock comprised of high composition of saturated fatty acids (28.2%) as compared to soybean oil (19.2%). In biodiesel derived from soybean oil, the precipitates were formed due to steryl glucosides. The formation of precipitates in the cottonseed oil derived biodiesel was attributed to both steryl glucosides and monoglycerides along with other minor components. Halim et al. [86] proposed the marine *Chlorococcum sp.*despite the low lipid content (7.1 wt%) to be suitable for the production of biodiesel on the basis of the lipid constituents (fatty acids) present in the feedstock. The oleic acid (C 18:1), a monounsaturated fatty acids was predominant in the feedstock that comprised of around 63 wt% followed by palmitic acid (C 16:0) comprising of 19 wt% and other constituents in minor amount. The feedstock comprising of both monounsaturated fatty acids and saturated fatty acids in a balanced proportion can be a good feedstock for biodiesel as it will best compromise between low temperature properties and oxidation stability of biodiesel.

The feedstock has been found to influence the exhaust particulate emissions from compression ignition engines. In most of the studies done so far, the exhaust emissions from biodiesel and biodiesel blends have shown reduction in CO, hydrocarbons (HC), and particulate matter emissions and enhancement in NO_x emissions. Biodiesel reduces soot emissions but increases soluble organic fraction of the particulate matter which has been attributed to its distillation characteristics and high viscosity [132]. Usually, the emission from the biodiesel is low as compared to that from mineral diesel except NO_x [15]. NO_x emissions is usually found to be more in biodiesel than from mineral diesel [134]. A higher emission of NO_x can pose a major disadvantage for the application of biodiesel as alternative fuel as NO_x leads to ozone formation in troposphere [133]. The neat biodiesel (100%) has been reported to emit 16% more NO_x than the mineral diesel [135]. However, a study by Sahoo et al. [136] has shown a lower NO_x emission (4% less) from neat biodiesel derived from polanga oil (*Calophyllum inophyllum* L.) as compared to mineral diesel at full load. The reason for a better performance by polanga oil biodiesel has been attributed to engine geometry, compression ratio, and lower temperature in the combustion chamber. A high flame temperature results in high NO_x emissions [137]. Fang et al. [138] reported that low temperature combustion of biodiesel which could be achieved by retarding injection timing (late injection). This can effectively reduce NO_x emission because a lesser thermal NO_x is formed. Glaude et al. [139] reported that the emission of NO_x from biodiesel and mineral diesel are comparable as they both contain double bonds. The fatty acid alkyl esters (biodiesel) comprises of unsaturated (double bonds) and saturated (single bond) fatty acids, whereas, mineral diesel contains aromatics and naphthenoaromatics (unsaturated species) among the hydrocarbons. The NO_x emission in biodiesel is enhanced by the presence of these unsaturated species. A high NO_x emission has been correlated with high flame temperature (T_f). Thus, among the biodiesel, the presence of large amount of unsaturated bonds leads to a high flame temperature (T_f), whereas, high content of saturated bonds leads to a smaller T_f. A lower T_f is usually observed with FAAE than with the mineral diesel (that comprises predominantly of hydrocarbons). The reason attributed is that the carbon atom in FAAE is bound to two oxygen atoms within a carboxylic group, thus not contributing to the heat of combustion. The generation of CO_2 molecule from the carboxylic acid present in the FAAE consumes sensible heat generated by the reaction products thus lowering the flame temperature [139].

Lackey and Paulson [140] reported a better performance of exhaust emissions from biodiesel obtained from the edible feedstock (soybean, canola) as compared to the feedstock of waste origin (yellow grease). A higher particulate matter (PM) emission rate was also observed from yellow grease derived biodiesel as compared to mineral diesel. It is reported that the mean mobility diameter of the PM emitted from biodiesel exhaust ranges from 27% smaller to 9% larger than that of diesel. The emission rate of elemental carbon (EC) and black carbon (BC) was found to be higher using yellow grease biodiesel as compared to soybean and canola based biodiesel. The reason with high emission rate of EC and BC may be the presence of burnt particulates in the yellow grease as the feedstock may have undergone polymerization, hydrogenation, or oxidation at high temperature. The findings placed biodiesel from soybean or canola to be advantageous as compared to that from yellow grease (obtained from waste oil) feedstock.

Surawski et al. [141] developed biodiesel from three feedstocks viz. soy, tallow, and canola and measured the concentration of particles, vapor phase polycyclic aromatic hydrocarbons (PAH); and reactive oxygen species (ROS) from the exhaust of biodiesel blended with mineral diesel (B20, B40, B60, and B80). The particulate matter of aerodynamic size 10 μm (PM_{10}) showed reduction of 43 and 92% with B20 and B100, respectively, from soy oil derived biodiesel. Whereas, the reduction was 58 and 88% for tallow oil with B20 and B80, respectively; and 65 and 88% for canola oil with B20 and B80, respectively. The fuel type and blend percentage significantly influenced the particle number size distribution. Though, the three biodiesel fuels were efficient in the reduction of the particle number concentration at larger mobility diameters (>200 nm), the concentration of nanoparticles emitted was increased significantly in case of smaller mobility diameters

(< 50 nm) for canola and tallow based biodiesel. The effect of the feedstock was observed to be more significant in case of reduction percentage in particle and vapor phase PAH amounting to a minimum of 16 and maximum of 84 for tallow based biodiesel. The reduction observed with canola based biodiesel was 'no change' to 62% decrease, whereas for the soy based feedstock, the difference was 4% increase to 59% decrease with the biodiesel blends. Similarly, the ROS concentration was reduced by 21% for B20 tallow and increased by 16% for B20 canola [141].

The exhaust emission from mineral diesel also contains mutagenic and carcinogenic polycyclic aromatic hydrocarbons that stick on the surface of particulates to a large extent and even to gaseous phase to a small extent. Moreover, with the economics being a major concern in the commercialization of biodiesel, unmodified vegetable oil that possesses a high viscosity are being diverted for production of biodiesel. This causes higher engine deposits than the modified vegetable oils [130]. Karavalakis et al. [142] reported higher hydrocarbon and PAH emissions from the feedstock comprising of high constituent of esters of saturated fatty acid. The saturated blends portion of biodiesel causes a low volatility resulting in incomplete vaporization and combustion of the fuel. Thus, the biodiesel derived from palm oil showed higher hydrocarbon emissions than that derived from rapeseed oil. On contrary, Çelikten et al. [143] reported similar performance of fuel properties from rapeseed and soybean derived biodiesel. The disparity in the performance of fuel due to the feedstock warrants for a meticulous selection of the oil or fats for the synthesis of biodiesel. A major concern with non-edible plant based oil feedstocks can be the emission of toxins present in the feedstock that may remain in the biodiesel if not removed during purification. Non-edible plant species are also known to possess toxins (phorbol esters in *J. curcas*, saponins in *M. indica*, cyano lipids in *S. triguga*) and the effect of these on the combustion of biodiesel has not been taken in consideration. Thus, the biodiesel fuel emissions from non-edible feedstocks should be tested for the toxins that are present in the virgin oil.

4.2. Characterization of biodiesel and their fuel properties

The utilization of biodiesel as a fuel has certain advantages and disadvantages. Among the advantages is its renewable nature, being readily available, high cetane number, biodegradability, better combustion efficiency and low emission of toxic constituents (viz. sulfur, aromatic content, and particulate matter). The disadvantages include a high viscosity as compared to mineral diesel, comparatively low energy content, poor cold flow properties (which makes it unsuitable for use in countries where cold climate prevails), low oxidation stability (needs antioxidants to be added), a high emission of oxides of nitrogen, compromise with engine performance (due to lowered torque as compared to mineral diesel) and a high price (mostly attributed to high feedstock cost) [144].

The characterization of the biodiesel is an important aspect for the adherence of the fuel to governing specifications. There are national as well as internationally accredited specifications for biodiesel quality. Several specifications for biodiesel standards include American Society for Testing and Materials (ASTM) D 6751 (USA); DIN 51606 (Germany); European Organization (EN 14214) and India (IS 15607) [61]. These specifications are internationally accepted for biodiesel. Various countries have their own specifications for biodiesel. Contaminants may enter biodiesel fuel via many ways and resulting in the fuel going off-specification. The quantification of the contaminants important in terms of preventing damage to the engines and voiding manufacturer warrantees. The characterization of biodiesel includes the FAAE content, physical properties (density, viscosity) and fuel properties (flash point, oxidation stability, cetane number and others). The biodiesel should meet the international specifications [145]. Nuclear magnetic resonance has emerged as a promising tool for the characterization of fatty acid methyl esters (FAME) in biodiesel [146]. Various researchers have utilized NMR as a fast and accurate tool to determine the extent of conversion of triglycerides to FAME. Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy has gained immense importance and has developed as an important tool in the characterization of biodiesel and biodiesel blends [124]. In NMR spectroscopy, commonly employed protons are ^{1}H or ^{13}C, though ^{31}P has been used in some of the studies by researchers. Flores et al. [147] studied the blends of various types of biodiesel obtained from castor, cotton, peanut, pinion, soybean, and tallow oils. With the ^{1}H NMR spectra, it was possible to determine the composition of biodiesel obtained from various feedstocks and also of their blend with mineral diesel. The ^{1}H NMR spectra of the biodiesel can be augmented for the analysis of correlation between viscosity and crystallization, (the two important parameters in the specification described by ASTM D6751) of biodiesel blends. Although there are several feedstocks available for the synthesis of biodiesel, their applicability as a fuel for substitution or blend with mineral diesel will be considered only after they adhere to the specified fuel properties. Hence, the fuel that is derived from a particular feedstock should not only be sufficient for large scale production but also fulfill the criteria specified by national/international specifications [148]. The fuel properties of biodiesel derived from several plants and animal based feedstocks, waste cooking and waste frying oils have shown compliance with the ASTM D 6751 specifications. The data on the fuel properties of biodiesel from microalgal oil is scanty and needs to be observed before they can be utilized as a transport fuel. Among the wide range of parameters that comprises of the fuel properties, the cold flow property and the oxidation stability of the fuel demands special attention because of their inverse relationship. The issue with microalgal oil is that it comprises of constituents other than triglycerides and free fatty acids viz. hydrocarbons, sterols, sterol esters, wax, and alcohol [49]. These constituents ought to influence not only its esterification and/or transesterification but also its fuel properties. Among these constituents, hydrocarbons are expected to enhance or supplement to the fuel properties of microalgal derived biodiesel but the same cannot be predicted for other constituents. The hydrocarbon content varies in the microalgal feedstock and is found to be very high in some of the species (viz. *B. braunii*).

Calorific value is an important parameter for the utility of a fuel in CI engine. The calorific value of methyl esters (biodiesel) is lower than the mineral diesel because of the oxygen content in the former that is helpful in complete combustion of the fuel in engine [149]. Kanda et al. [150] reported the calorific value of lipid extracted from blue-green microalgae (*Microcystis aeruginosa*) to be 33.8 MJ kg^{-1}. However, the gross calorific value and net calorific value of biomass of *Chlorella* sp. was found to be comparably low (18.59 ± 0.42 and 15.88 ± 0.39 MJ kg^{-1}). With a fixed carbon content of 15.08 ± 1.21 wt% and elemental carbon content of 47.54 wt%, the de-oiled cake of the microalgae was proposed to be suitable for thermo-chemical conversion (via pyrolysis) to biochar and bio-oil [151]. Ahmad et al. [21] reported a high heating value from the bio-oil obtained from microalgal oil (29 MJ kg^{-1}) as compared to that from wood (21 MJ kg^{-1}). Though, the stability of the bio-oil obtained from microalgae was less as compared to the fossil oil, it was higher than that obtained from the wood. Thus, microalgal oil has been proposed to be a better option for fuel oil than the lingo-cellulosic material (wood). Sydney et al. [152] found that the biodiesel developed from the microalgae, *B. braunii* LEM 14 comprised predominantly of palmitic, C16:0 (21%) and oleic acetate, C18:1 (47%) followed by steric,

Table 8
Calorific value of biodiesel from some microalgae and plant oil.

Microalgae/Plant	Source	Calorific value (MJ kg^{-1})	Reference
Microalgae			
M. aeruginosa	Freshwater lake	HHV = 33.8	[150]
Chlorella sp.	Manmade lake	GCV = 18.59	[153]
	NCV = 15.88		
Chlorella protothecoides	Culture collection of Alga, University of Texas, USA	HHV = 41	[154]
Plant *P. dactylifera* (Date seed)	–	HHV = 39.55	[30]
Sunflower	–	HHV = 45.3 ± 1.2	[103]
Pongamia	–	HHV = 40.216	[147]

HHV = Higher heating value.
GCV = Gross calorific value.
NCV = Net calorific value.

C18:0 (4%); linolenic, C18:2 (7%); and α-linolenic acetate, C18:3 (8%). Table 8 depicts the calorific value obtained from microalgae and plant species [30,103,150,153–155].

The maximum water content in biodiesel is specified not to exceed 500 mg kg^{-1} by ASTM D6751 specifications. As the esterification reaction results in the production of water, scope for biodiesel to become contaminated with water. This may lead to the fuel going off-specification during storage. The presence of water may also promote the growth of microbes in the storage tanks which could lead to corrosion of metallic parts with which the fuel comes into contact. The presence of water may further lead to formation of sludge and slime which could result in blockage of fuel filters and fuel lines thereby damaging vehicles fuel injection systems. The solubility of water is dependent on the temperature and composition of fuel [156]. Wu et al. [157] reported biodiesel obtained from *Trichosporon capitatum* to have a better cold filter plugging point (CFFP). While, the CFPP for biodiesel from *T. capitatum* was −15 °C, the values reported for soybean, olive, high oleic sunflower, rapeseed, and corn oil were −5, −6, −6, −10, −12, respectively. This has been attributed to a high concentration of unsaturated fatty acids {total 89% that comprised mainly of oleic acid (80%)}. Though, the biodiesel possessed a good low temperature property in terms of CFPP, the fuel may bear a low oxidation stability due presence of high unsaturated fatty acid alkyl esters.

5. Sustainable approaches for biodiesel production from plant oil

Plants and trees that bear seed or kernels are usually grown worldwide citing their efficiency to convert carbon dioxide to oxygen. Many countries have minimum criteria to have a dedicated portion of land for forestation. India, South Africa, Brazil, and several other countries have significant landmass occupied by forestry. Given, the stringent environmental laws that many countries follow, efforts to increase the land under forestry are underway. Many of the wild trees have the potential to produce oil in their seed and kernels. Furthermore several of these oil producing trees are non-edible as they contain toxins. Thus, the oil from these plants and trees offer an easy mode of access for its utilization as the feedstock in the synthesis of biodiesel. They can be easily planted in the unutilized arable land (e.g. adjacent to railway tracks), and/or un-arable land (by enhancing their productivity through scientific methods). Once grown as a plant or tree, they can provide dual benefit of oil as feedstock (for biodiesel) and fixation of carbon dioxide (a greenhouse gas), aside from providing scenic beauty and conservation of natural resources. Monoculture of plant species has been explored to provide an easier route for the collection of the seeds. The most commonly used feedstock that has become synonymous with biodiesel is *J. curcas*. This feedstock has often been cultivated in the form of monoculture. Recently, there have been advancements to enhance the lipid content in the seeds by genetic engineering. The monoculture of the plants suitable for a high lipid yield of fatty acids (prone to esterification and transesterification) can also be grown as mixed crop (with the edible crops) or separately in wastelands.

Another approach in the production of biodiesel is its synthesis at room temperature. Many tropical nations like Sub-Saharan African and Asian countries have tropical climates where room temperatures can go beyond 30 °C. Though, the synthesis of biodiesel is an endothermic reaction, some of the feedstock has shown their conversion to FAAE at comparable rate. The purification of biodiesel is almost a mandatory step to meet the stringent international specifications. The reason for the purification of biodiesel can be attributed to formation of side products such as soap, water and emulsified products. The catalysts also play an important role in the methods and purification steps that is desired to meet the specifications for biodiesel. The homogeneous catalyst despite their high activity results in loss of the products (FAAE) through repeated washing. Heterogeneous catalysts offer a high catalytic activity even in presence of FFA and water and could lead to easy separation of glycerol from biodiesel are preferable [158].

5.1. Cultivation of plant crops in wasteland

The sustainability of biodiesel as a renewable fuel can be achieved if the feedstock crops are grown on wastelands. Ruan et al. [159] studied the growth of *Datura candida*, *Xanthium sibiricum*, *Kosteletzkya pentacarpos*, *Hibiscus trionum*, and *Rhus typhina* on unproductive agricultural lands to produce biodiesel. All the plants except *R. typhina* were found to be suitable for cultivation on the basis of their seed oil content (ranging from 17.5% for *H. trionum* to 41.9% for *X. sibiricum*). The plant, *R. typhina* possessed a low oil content of 9.7% and was reported unsuitable for cultivation. Plants that have a good potential for growth and oil productivity in the seed or kernels can be planted on the unutilized lands. Misra and Murthy [160] reported that 33 million hectares of wasteland in India could be utilized for the production of biodiesel. Growing *J. curcas* on the wasteland, barren, and underutilized land could provide a large scale production of feedstock for biodiesel. Azam et al. [61] projected that utilizing wastelands for the cultivation of plant crops *Azadirachta indica* and *P. pinnata* on 40.96 and 19.9 million hectares of land would be sufficient to meet the target of 100% replacement of imported biodiesel in India that amounted to 87.5 million tons in 2003–2004. Among the feedstocks, *J. curcas* is considered to be a multipurpose crop as it can be used in fencing as well as utilizing its seeds for extracting oil [161]. Biswas et al. [151] emphasizes

that around 4 million hectares of wasteland along the railway tracks, roads, and canals can be utilized for the plantation of *J. curcas* in India. It is estimated that cultivation of *J. curcas* on 10 million hectare of wasteland could yield oil that could produce 5 million tones of biodiesel. Though, concerns have been raised on the utilization of wastelands. Findlater et al. [162] states that there could be local impacts on the poor and marginal communities that depend on these 'wastelands'. As the plantation of *J. curcas* on the wastelands may continue for a long time period, any adverse environmental impact on the soil ecosystem or indirect impact on mankind will have a profound effect. The effect of *J. curcas* on the wasteland have been identified as low seed yield and loss of fodder for the animals. Kumar et al. [163] reported that the wastelands identified for cultivation of *J. curcas* in India was being used for marginal farming by landless laborers, and as grazing ground. Some of the identified wasteland was found to be remotely located. Montobbio and Lele [164] also report that the information on the region specific field performance of *J. curcas* on the identified wasteland is very limited.

5.2. Room temperature synthesis of biodiesel

The synthesis of biodiesel at room temperature offers a good opportunity to decrease the energy input in the production of biodiesel. As the synthesis of biodiesel is an endothermic process, the tropical countries will be naturally more suited for the room temperature of biodiesel. Sharma et al. [165] reported room temperature (35 °C) synthesis of biodiesel from waste frying oil using homogeneous catalysts (NaOH and CH_3ONa) and methanol as reactant. Pramanik et al. [166] reported on room temperature (25 °C) for the synthesis of biodiesel whereby an acid catalyst, HZnPS-1 was found to give FAAE yield of 81.5 wt%, though the extent of conversion is low when compared with the EN specification (min. value of 96.5%). The reason for the low FAAE yield may be as a function of the reaction being done at the comparatively low temperature (25 °C) for 24 h with 5 wt% catalyst. Co-solvents have been found to be effective in enhancing the transesterification rate when the reaction temperature is comparatively low. Guan et al. [167] utilized soybean oil for synthesis of biodiesel at 25 °C in a closed batch reactor using various co-solvents (dimethyl ester, diethyl ester, *tert*-butyl methyl ether and tetrahydrofuran) with KOH as catalyst. The reaction was complete in 20 min using the co-solvent, whereas, in the absence of a co-solvent only 78% conversion could be achieved. It was observed that performing the reaction in a microtube reactor system could enhance the mass transfer. Sun et al. [168] performed room temperature (25 °C) synthesis of soybean oil using Ca_4SiO_6 as solid base catalyst. The yield of FAAE was comparatively low (ca. 80%) with a 10 h reaction time. When subjected to a higher temperature (methanol reflux), the FAAE yield was increased to 95% in 2 h reaction time. When NaOH (a homogeneous catalyst) was used, high FAAE yield was obtained. Hence, temperature is an important parameter in obtaining a high conversion of feedstock to biodiesel and depends on the catalyst used in the reaction.

5.3. A hybrid feedstock for synthesis of biodiesel

With scarcity in feedstocks, a hybrid feedstock can be one of the solutions to support a biodiesel industry. A blend of feedstocks can offer the advantage of a better quality of biodiesel with desired fuel properties viz. enhanced oxidation stability, better cold flow properties, and a lowered corrosion to engine parts. Few researchers have utilized hybrid feedstocks for the synthesis of biodiesel. Qiu et al. [102] used edible feedstocks (soybean and rapeseed oil) to derive biodiesel using NaOH as a catalyst. The acid value of soybean oil and rapeseed oil was 0.9762 and 1.631 mg KOH g^{-1}, respectively, which made the single step transesterification feasible for the feedstocks. However, the second generation feedstocks (non-edible) usually have a high acid value (>4.0 mg KOH g^{-1}) and thus either two step process {comprising of esterification (with homogeneous catalyst) followed by transesterification} or a single step esterification (utilizing a solid acid catalyst with high catalytic activity) is adopted. Sharma and Singh [70] utilized *P. pinnata* and *M. indica* in a 50:50 ratio and found a similar yield and conversion of biodiesel as compared to that of the individual feedstocks in a two-step process viz. esterification with H_2SO_4 followed by transesterification with KOH as base catalyst. Ethier et al. [169] utilized chicken fat and soybean oil mixture in 50:50 ratios for synthesis of biodiesel. A similarity between biodiesel and mineral diesel is their multi-component composition. While, the mineral diesel comprises of a mixture of hydrocarbons; biodiesel comprises of a mixture of fatty acid alkyl esters. Nogueira et al. [81] blended babassu seed oil with cotton seed and babassu seed oil soybean oil separately to obtain biodiesel. The density of biodiesel obtained from babassu oil was lower than that obtained from cotton seed and soybean oil, because of shorter chain length in the former. The viscosity of the babassu oil biodiesel was also lower than that of cotton seed and soybean oil biodiesel due to low density. Though, the conversion of the biodiesel has not been reported, it was found that the density of the biodiesel obtained (from the mixture of feedstocks) theoretically correlated with the experimental values with a relative difference of $<0.21\%$. Chen et al. [170] utilized a blend of soapnut oil methyl ester (SME) and jatropha oil methyl ester (JME) to obtain a better fuel quality of biodiesel. The SME contains high percentage of monounsaturated fatty acids that imparts good oxidation stability. Whereas, JME contains a large amount of unsaturated double bonds that renders the fuel to poor oxidation stability. Hence, in order to get biodiesel with improved fuel properties, the biodiesel were blended. The blend ratio of 35:65 (by weight) of SME: JME was observed to be improve the cold filter plugging point and oxidation stability of the biodiesel. With the use of biodiesel derived from two different feedstocks the blend of biodiesel with diesel up to B40 was found to show satisfactory fuel properties. Meneghetti et al. [171] used mixture of three vegetable oils (cottonseed, soybean, and castor) for the synthesis of biodiesel. It was observed that blend improved the purification process. Castor oil possessed a high viscosity (225.8 $mm^2 s^{-1}$ at 40 °C) which may be a deterrent in its application as a neat biodiesel hamper. However, it offers an excellent lubricity and its blend with other feedstock may enhance some of the characteristics of the biodiesel.

5.4. Continuous transesterification for production of biodiesel

The biodiesel industry will rely on continuous production of biodiesel because of its operational benefits and easy implementation at industrial scale. Several researchers have proposed continuous production of biodiesel. An excellent review on the continuous production of biodiesel has been described by Qiu et al. [172] where various process intensification technologies in the synthesis of biodiesel have been discussed in detail. Reactors that provide continuous production of biodiesel included static mixers, micro-channel reactors, oscillatory flow reactors, cavitational reactors, rotating/spinning tube reactors, and microwave reactors. Membrane reactors, reactive distillation, and centrifugal contractors offer simultaneous reaction and separation of the products obtained after transesterification. It has been reported that new processes for the synthesis of biodiesel reactor rate are enhanced by intensifying the transport process and enhanced mixing between alcohol and oil. A continuous transesterification for production of biodiesel has been described by He et al. [173] using anion-exchange resin D261 as catalyst in a fix bed reactor. However, the biodiesel which comprises of FAME constituted only

90% up to a run time of 4 h and decreased to 23.7% in 8 h. This shows that catalyst has to be added continuously to make the production of biodiesel up to the desired level [173]. Kwon et al. [174] demonstrated a non-catalytic thermo-chemical process as a continuous system for simultaneous esterification and transesterification of animal fat comprising of beef tallow and lard utilizing charcoal as a porous material and CO_2. A conversion of 98.5 ($\pm$0.5)% was achieved in only 1 min, though a comparatively high temperature (350–500 °C) was used for the synthesis of biodiesel. CO_2 has been hypothesized to cause dissociation of bonds at high temperature and impeding the reversible reaction during transesterification. Hsieh et al. [121] used a packed bed reactor for the continuous production of biodiesel from soybean oil. A high yield of biodiesel (95%) was obtained in 168.2 min catalyzed by a solid base catalyst $\{Ca(C_3H_7O_3)_2/CaCO_3\}$. Santana et al. [18] utilized supercritical alcohol (ethanol) and solvent (carbon dioxide) for the continuous production of biodiesel using a solid acid catalyst. The advantage of this process was the formation of biodiesel in a short reaction time (6 min). However, the disadvantages were the high consumption of energy as a high temperature (150 to 200 °C) and pressure (200 bar) along with a high molar ratio of vegetable oil to ethanol (1:25) were taken. The optimum yield of biodiesel obtained was comparatively low (80%) and the FAAE constituent were also $<90\%$. da Silva et al. [175] used continuous production of biodiesel from soybean oil by transesterification in supercritical conditions. The yield of FAAE obtained was found to be higher in a microtube reactor (inner diameter 0.76 mm) as compared to that obtained from tubular reactor (inner diameter 3.2 mm). This was likely due to better mass transfer being facilitated inside the microtube reactor. The use of CO_2 as co-solvent was found not to enhance the yield of FAAE significantly. The operating conditions for the reaction were: temperature, 325 °C; pressure, 15 to 20 MPa; and ethanol to oil molar ratio, 40:1 and 20:1. As a high reaction temperature was subjected, a small amount of fatty acids (<5.0 wt%) was decomposed. Hu et al. [176] demonstrated a continuous flow integrated process for the development of biodiesel from rapeseed oil using KF loaded on CaO (KF/CaO) as a solid base catalyst. The contact area of gas–liquid is larger than that of liquid–liquid and thus has been projected to provide better biodiesel production efficiency. The continuous process comprised of three fluidic modules and one rectification column. Each fluidic module comprised of three zones. The upper zone augmented condensation of methanol vapor, the middle zone comprised of a microporous solid base catalyst, and the lower zone utilized an online separator for the separation of biodiesel and glycerol. The KF/CaO was found to work for 144 h with easy and automatic separation of biodiesel and glycerol thus overcoming the requirement of washing with water.

Behzadi and Farid [112] reported on a process for continuous production of biodiesel from fats and oils with a high reaction rate and less cleaning than required for crude biodiesel. The fats and oil were atomized to provide a larger surface area allowing better contact of oil and methanol which facilitated a high reaction rate thus reducing the reaction time to few seconds. Atomization of the oil/fat was done using high pressure diesel injection pump which resulting in a droplet size of 100–200 μm. The atomized oil/fat was dispersed in a reaction chamber that contained vapors of methanol in a counter current flow arrangement. They found that a high reaction temperature lower than the degradation temperature of the oil (i.e. ≤ 200 °C) can be utilized for esterification/transesterification. Thanh et al. [177] used an ultrasonic reactor for the continuous production of biodiesel from waste cooking oil (WCO). Two step transesterification was adopted whereby WCO and alcohol was added at difference molar ratios and catalyst amount. WCO and methanol were added to the reactor by piston and peristaltic pump, respectively. The step wise addition of methanol led to a faster separation of the glycerol from biodiesel. As the density of glycerol (1.26 g cm^{-3}) is much higher than that of methanol (0.79 g cm^{-3}), a high amount of methanol in the glycerol lowers the density of the mixture thus rendering their separation difficult. The optimized molar ratios (methanol to WCO) in the first and second step were 2.5:1 and 1.5:1, respectively. The amount of catalyst loaded in the first and second step was 0.7 and 0.3 wt%, respectively. Ultrasonic irradiation augments the formation of fine emulsions of immiscible liquids (oil and alcohol) which increases the interface area between methanol and oil. Thus, a short residence time (<1 min) of the reactants in the ultrasonic reactor in both the two steps was required. The total time for the completion of the reaction, separation and purification took 15 h. Phan et al. [178] designed oscillatory baffled reactors for continuous production of biodiesel. The reactors varied in baffle design viz. central sharp edge baffles, helical shaped baffles, and axially oriented sharp-edged helical baffles. The baffled reactors offered plug-flow of the immiscible liquids (oil and alcohol) at lower oscillatory Reynolds numbers. The central sharp edge baffles and helical shaped baffles suffered a few limitations. Homogeneity was not found in central sharp edge baffles designed reactor. A better but still incomplete homogeneity was observed in helical shaped baffle reactor that resulted in segregation of the biodiesel rich phase and the glycerol rich phase. A greater shear force at the baffle edge offered a more homogeneous mixture (by effective mixing of the two phase) in sharp-edged helical baffle reactor. The non-catalytic continuous transesterification of refined palm oil has been proposed by Joelianingsih et al. [179] in a bubble column reactor (BCR). The BCR has been proposed to initiate reaction and distillation in a single step. The products formed were continuously removed, while the oil was retained in the reactive zone. The products formed were in gaseous phase, whereas, the reactant (oil) was in the liquid phase. The yield of biodiesel enhanced with the increase in methanol feed flow rate and reaction temperature. Fatty acid methyl ester (biodiesel) yield of 94% has been reported in 240, 180, and 90 min at reaction temperature of 250, 270, and 290 °C. However, the high reaction temperature (250–290 °C) for the production of biodiesel ought to make the production cost energy intensive.

Ren et al. [180] used a fixed bed reactor packed with an anion-exchange resin (D261) as a heterogeneous catalyst for continuous production of biodiesel using co-solvents (methyl *tert*-butyl ether, *n*-hexane, *tert*-butyl alcohol, and tetra-hydrofuran). Methanol, oil, and co-solvent (*n*-hexane) were pre-heated prior to their injection in the reactor using a peristaltic pump. The addition of *n*-hexane as co-solvent resulting in the reactants becoming homogeneous thereby improving the reaction rate and resulting in a high conversion of FAAE. The advantage this process was regeneration of the catalyst in-situ. The regeneration of the catalyst was carried out in a three step process which involved flushing with methanol (flow rate 10 mL min^{-1} for 20 min to remove adsorbed organics viz. glycerol), washing with 5.0 wt% dissolved in methanol (flow rate 1.5 mL min^{-1} for 90 min to restore the activity of resin), and again washed with methanol to neutralize the pH. Apart from acting as an active catalyst, the anion-exchange resin also adsorbed glycerol resulting in a pure FAAE. Chongkhong et al. [181] proposed the use of a continuous stirred tank reactor (CSTR) followed by purification of the products for the continuous esterification of palm fatty acid distillate. The biodiesel yield (97%) of the continuous process was lower than the batch process (99%). However better conversion (FAAE of 97.3%) was achieved in comparison to batch process where the FAAE content was 93.9% before purification.

Continuous processes have been proved to be a great tool while using biocatalysts for biodiesel production. Lipases are known for their inactivation by short chain alcohols and glycerol. The continuous

process provides a solution for lipase inactivation by alcohol whereby alcohol can be easily added in step wise manner and co-solvent synthesis can be effectively carried out. Glycerol inhibition is addressed as glycerol can be continuously removed. Continuous process using both extracellular and intracellular lipases has been reported by researchers [182–184]. Methanolysis of soybean oil by immobilized lipase (Novozyme 435) in *tert*-butanol was carried out in packed bed reactor. Optimized reaction conditions of flow rate 0.1 mL min^{-1}, 52.1 °C and methanol to oil molar ratio of 4:1 gave 82.81 ± 0.98% biodiesel conversion. No decrease in activity of Novozyme 435 was seen for 30 days continuous operation of packed bed reactor [185].

6. Sustainable approaches for biodiesel from microalgal oil

Synthesis of biodiesel has gathered immense interest worldwide. However, their viability as an alternative and renewable fuels need to be justified in terms of their sustainability, availability of adequate oil and techno-economics. The cultivation of microalgae is cost intensive at present and several efforts have been made to lower the cost, making the production cost of biodiesel feasible. Many researchers have adopted an optimistic view on microalgal biofuels. Chisti [186] stated that microalgal biodiesel seems to be the only renewable fuel that has the potential to completely replace the mineral derived liquid transport fuel. However, its cost should be reduced to one-tenth the current cost in order to make it competitive. The approach towards improved sustainability of biodiesel derived from microalgal oil includes selection of a suitable species that could be cultivated with minimal energy inputs. The dry biomass and lipid productivity should be high. Energy inputs can be decreased by recycling the water, using natural light, and applying the best techniques for the cultivation and extraction of oil. Microalgae can be grown using municipal and industrial wastewater thus leading to environmental sustainability. In an attempt for their long term sustainability, several approaches that can be considered sustainable have come up [20,49,187].

6.1. *Scope of wastewater as a nutrient medium for the cultivation of microalgae*

The treatment of wastewater arising from several industries is a major concern and requires large energy inputs. The non-compliance of the treatment of the wastewater also results in polluting the river and the aquatic fauna therein. The culture of the microalgae offers a dual benefit of treatment of wastewater and low cost culture of microalgae thus saving on the nutrient dose [187]. The cost of tertiary wastewater treatment for the removal of residual nitrogen, phosphorous, and heavy metals requires large infrastructure and bears a high cost. Abdel-Raouf [188] reported that the cultivation of microalgae using wastewater as a substrate could be a solution to tertiary and quandary treatment of wastewater with the added benefit of biomass production that can be used for several purposes. The microalgae can remove biochemical oxygen demand (BOD), nitrogen, phosphorous, heavy metals and have inhibited the growth of coliforms. The presence of nitrogen and phosphorous in wastewater can be used as a source of nutrient for the production of biodiesel. Subashchandrabose et al. [189] reported that microalgae utilize ammonium, nitrate, and phosphate as nutrients. These are common constituents present in wastewater. Microalgae grown using wastewater can serve the dual purpose of wastewater treatment as well as reduction in the cost incurred in the culture of microalgae [187].

Wu et al. [190] utilized industrial wastewater from industrial park for the culture of *Chlamydomonas* sp. The lipid profile of the fatty acid produced were predominantly palmitic acid (61.1%) followed by linolenic acid (21.1%), stearic acid (6.7%), palmitoleic acid (6.2%), and myristic acid (4.9%) after a 10 days culture period. Though, the content of linolenic acid was low (10.2 and 11.1%) in the 6th and 8th day of the cultivation, respectively, the high value of linolenic acid (21.1%) obtained on the 10th day of the cultivation poses a concern and thus will have to be harvested by the 8th day to ensure the presence of linolenic acid remains within the acceptable limits of European Standards (EN 2004). Mohan and Devi [191] utilized acid rice effluents discharged from biohydrogen production facility (from food waste products) which served as a carbon sources for the microalgae. Volatile fatty acids (which comprises of carbon chain backbones) are generated during biohydrogen production supports microalgal biomass production as well as lipid accumulation. Lipid and biomass productivity was compared using both synthetic volatile fatty acids and fermented fatty acids from the effluent as carbon source. The lipid content of the microalgae which comprised of a mixed culture obtained from a lentic water body receiving domestic effluents was 26.4% while the biomass production was 1.42 ± 0.02 mg mL^{-1} for the fermented fatty acids. The treatment of the wastewater was also observed while cultivation of microalgae with fermented fatty acid, and the chemical oxygen demand (COD) removal efficiency up to 91.4% was observed. Xin et al. [16] used *Scenedesmus* sp., for the production of biodiesel utilizing secondary treated wastewater effluent as a source of nutrients. A high removal efficiency of 98% with respect to inorganic nutrients was observed in 10 days. The lipid accumulation productivity had a maximum value of 0.008 g L^{-1} d^{-1} on the 10th day. Zhang et al. [192] studied the efficiency of wastewater treatment during the heterotrophic cultivation of *Chlorella pyrenoidosa* and the impact of bacteria on the productivity of algal biomass and lipid content. Though, the coexistence of bacteria with microalgae had insignificant effect on productivity of biomass in the latter, the total lipid content in algae and lipid production rate were slightly reduced. The reason attributed to the decreased lipid content and lipid productivity was the competing nature of bacteria with microalgae for nutrients. Li et al. [193] investigated the efficiency of various strains of *C. vulgaris* for the treatment of citric acid effluent and biomass production. An increasing tendency in the peak average specific growth rate of *C. vulgaris*, C9-JN2010was observed upon dilution of citric acid effluent (0.6 ± 0.01, 0.74 ± 0.02, and 0.98 ± 0.04 g L^{-1} at 10, 15, and 20% dilution, respectively) in 5 days of cultivation. The dry biomass content began to decrease at higher concentration of citric acid effluent and was 0.84 ± 0.03, 0.67 ± 0.01, and 0.56 ± 0.01 g L^{-1} at 40, 60, and 80%, respectively, in 5 days of cultivated period. The removal of COD, BOD, total organic carbon, nitrogen, and phosphorous was > 90%. The polyunsaturated fatty acid constituent in the extracted lipids was highest (i.e. 73.4%) followed by saturated fatty acids (22.5%) and monounsaturated fatty acids (3.5%).

Martinez et al. [194] utilized pretreated wastewater obtained from submerged membrane bioreactor for the culture of microalgae. A semi-continuous mode of cultivation of microalgae (mixed culture) resulted in biomass productivity of 234 mg L^{-1} d^{-1} operated over 42 days. A high nutrient removal efficiency of 97.8 ± 3.9% was observed for phosphate (PO_4^{-3}-P) and 67.2% for ammonium (NH_4^+-N) which were utilized by microalgae by transformation into biomass. The pH was maintained at 7.2 to ensure that the nitrogen and phosphorous removal was only due to growth of biomass and not due to ammonia stripping which occurs at $pH > 9$. Riaño et al. [195] reported 70% removal of the total chemical oxygen demand (TCOD) and phosphate by growing microalgae in a photobioreactor with wastewater obtained after processing of fish. Chinnasamy et al. [80] utilized wastewater effluent from carpet industry in combination with the municipal sewage water for cultivation of microalgae to be utilized as

Table 9
Wastewater utilization in cultivation of microalgae.

Source of WW	Wastewater Quality	Microalgal species	Removal of, nutrients	Lipid accumulation	Reference
Secondary effluent from domestic wastewater treatment plant	$COD=24 \pm 1.0$ mg L^{-1} $TN=15.5 \pm 1.1$ mg L^{-1} $TP=0.5 \pm 0.01$ mg L^{-1} NH_4-$N=2.5 \pm 0.01$ mg L^{-1} $pH=7.7 \pm 0.02$	*Scenedesmus* sp.	98% (of inorganic nutrients in 10 days)	31–33% of DW	[20]
Dairy wastewater	$COD=1195 \pm 7.0$ mg L^{-1}, $TN=118.0 \pm 2.8$ mg L^{-1}, $PO_4^{-3}=149.0 \pm 2.8$ mg L^{-1}, NH_3-$N=48 \pm 1.5$ mg L^{-1}, Total solids$=1490 \pm 14$ mg L^{-1}, $pH=8.3 \pm 0.2$	*Chlorella* *Zofingiensis*	97.5% of PO_4^{3-}, 51.7% of TN, (CO_2 regulation); 42.0% of PO_4^{3-}, 79.6% of TN, (HAc regulation)	31.8%	[33]
Mixture of carpet industry effluent (85–90%) & municipal Sewage water (10–15%)	COD=1412 (UT) COD=106–183 (T) BOD=331–487 (UT) BOD=2–21 (UT)	Consortium of 15 microalgae including: Freshwater (*Botryococcus braunii*, *Chlorella saccharophila*) & Marine water (*Dunaliella tertiolecta*, *P. carterea*)	$>96\%$	in 72 h	$6.82 \pm 0.08\%$
Untreated industrial wastewaters Taichung Science Industrial Park, Taiwan	Conductivity$=2.43$ μS cm^{-1} $COD=42.2$ mg O_2/L NH_4^+-$N=38.4$ mg N/L NO_3^--$N=3.1$ mg N/L Organic $N=16.2$ mg N/L PO_4^{3-}-$P=44.7$ mg P/L	*Chlamydomonas* sp.	100% for NH_4^+-N & NO_3^--N 33% for PO_4^{3-}-P	18.4% of DW	197
Citric acid effluent	$COD_{Cr}=13000 \pm 1000$ mg L^{-1} $BOD_5=6000 \pm 500$ mg L^{-1} $BOD_5/COD=0.6$ 0.05 $TOC=800 \pm 50$ mg L^{-1} NH_x^+-$N=60 \pm 5$ mg L^{-1} $TN=300 \pm 20$ mg L^{-1} $TP=30 \pm 5$ mg L^{-1} $TSS=0.4 \pm 0.05$ g L^{-1} pH=4.5–5.5	*C. vulgaris* BOD > 90%	COD > 90%	340 mg g^{-1} of DW	[200]
Treated domestic sewage	$Na^{2+}=28.98$ mg L^{-1}, $NH_4^+=12.76$ mg L^{-1}, $K^+=7.99$ mg L^{-1}, $Mg^{2+}=2.63$ mg L^{-1}, $Zn^{2+}=0.71$ mg L^{-1}, $NO_3^-=0.2$ mg L^{-1}, $PO_4^{-3}=2.00$ mg L^{-1}, $Cl^-=1.70$ mg L^{-1}, $SO_4^{-2}=4.21$ mg L^{-1},	20 strains of microalgae	79.63% of nitrogen, phosphorous	36% of DW	[159]

MS=Microalgae species.
DW=Dry weight.
HAc=Acetic acid.
TN=Total nitrogen.
TP=Total phosphorous.
COD=Chemical oxygen demand.
BOD=Biochemical oxygen demand.
UT=Untreated.
T=Treated.

feedstock for biofuel. A high nutrient removal of 96% was observed with a consortium of fifteen microalgal species. The consortium of microalgal isolates along with four microalgal species two belonging from freshwater (*B. braunii*, *Chlorella saccharophila*) and other two were from marine (*Dunaliella tertiolecta*, *Pleurochrysis carterae*) environment. It was found that both the treated and untreated wastewater from carpet industry supported the growth of both marine and freshwater microalgae. The reason attributed to the adaptability of marine species in wastewater has been the osmotic adjustment and regulation mechanism to tolerate hypo osmotic stress conditions by the marine microalgae. Among the studied species, all except *D. tertiolecta* showed a better growth rate in treated as well as untreated wastewater. The biodiesel production from the microalgal biomass has been projected to be $9.2-17.8\ \mathrm{t\ ha^{-1}\ yr^{-1}}$. Around 63.9% of the algal oil was found to be converted to biodiesel [80]. Table 9 describes the utility of wastewater in the cultivation of microalgae and removal of nutrients.

6.2. Carbon sequestration potential of microalgae

Carbon dioxide is the major contributor to the global warming [196]. CO_2 is a source of carbon for photosynthesis and to enhanced oil and natural gas recovery, production of urea, and for carbonation of food and beverages. Using photosynthetic microorganisms as a sink for carbon dioxide offers an attractive alternative as their efficiency to sequester CO_2 is three to five folds higher than the plants [197]. As the microalgae can grow faster than the terrestrial plants, the efficiency to fix CO_2 can range from 10 to 50% [198]. The content of CO_2 in water depends on the thermodynamics and mass transfer [199]. The CO_2 that can be effectively sequestered in aquatic environments by microalgae is limited by the Henry′s Law. Thus, temperature of the liquid and partial pressure of CO_2 above the liquid play an important role in sequestration of CO_2. While, most plants can accumulate carbon dioxide up to 0.1%, microalgae have the ability to accumulate up to 12% CO_2 at 35 °C [199]. Sánchez et al. [200] reported increase in the cell growth rate of 20% when the CO_2 supply was at low rate, though the authors have not mentioned the CO_2 supply rate.

The thermal power plants emit flue gases that contain up to 13% CO_2 [201]. Flue gases from power plants and industrial exhaust gases provide a potential carbon source for the cultivation of microalgae thereby accomplishing CO_2 bio-fixation to some level [202]. However, the capture of CO_2 from these sources is challenging and energy intensive. On-site microalgal cultivation is thought to be technically feasible and economically viable. However, microalgal CO_2 tolerance varies for different species. Ho et al. [198] reported that the CO_2 fixation by microalgae positively correlate with their cell growth rate. Some strains of microalgae are inhibited by CO_2 if NO_x are also present. Some species have been found to be tolerant of high temperature, high CO_2 concentration, and presence of toxic compounds (NO_x and SO_x). These include *Nannochloris* sp., *Nannochloropsis* sp., and *Chlorella* sp. While, *Nannochloris* sp., and *Nannochloropsis* sp. have shown CO_2 tolerance of 15%, various *Chlorella* sp. have shown tolerance to CO_2 varying from 15 to 50%. The CO_2 fixation ability of these species ranges from 500 to 1800 $\mathrm{mg\ L^{-1}\ d^{-1}}$. It is suggested that enhancing the light might increase the efficiency of microalgae in fixation of CO_2.

Valdes et al. [203] advocates for evaluation of CO_2 fixation efficiency by microalgae cultivated in a photobioreactor. They found that CO_2 content in water was found to be closely correlated with the photosynthetic activity of the algae. The stripping of CO_2 was found to be influenced by airflow rate in the photobioreactor. The injection of CO_2 occurred when pH increased to 8. The sequestration of CO_2 then leads to an increase in pH. Kaiwan-arporn et al. [79] reported the inlet CO_2 supply of 8% concentration to be the optimum amount for its fixation by the cyanobacteria, *S. aquatilis* at the rate of 5.3 $\mathrm{g\ L^{-1}\ d^{-1}}$ in airlift photo-bioreactor. One of the issues pertinent to carbon sequestration is the low mass transfer of CO_2 in water and the sensitivity of microalgal species to high CO_2 concentrations [204]. High CO_2 concentrations induce low pH in the culture medium reducing the activity of extracellular carbonic anhydrase which is responsible for the concentration of carbon in microalgal cells [205]. Honda et al. [206] optimized the carbon capture rates for *C. vulgaris*, *B. braunii* and *Spirulina platensis* cultivated using sewage sludge as a nutrient source. The carbon capture rate and biomass productivity were optimum when the hydraulic retention time and solids retention time were 1 and 18 days, respectively. The production rate of microalgae and CO_2 capture rate were found to decrease when starved of phosphorous. The CO_2 supply and availability of micronutrients were assumed to be the factors affecting the optimal growth of the microalgal species. The improvement of CO_2 diffusion in water for uptake by microalgae can be enhanced by the use of micro-bubble diffusers [206]. The accumulation of CO_2 in microalgae may lead to the production of protein or lipids. The production of lipids is desirable in terms of biodiesel production. Sydney et al. [207] reported the fixation rate of CO_2 by *D. tertiolecta*, *C. vulgaris*, *S. platensis*, and *B. braunii* resulted biomass yields of 496.98, 318.61, 272.4, 252.64 $\mathrm{mg\ L^{-1}\ day^{-1}}$, respectively. Though, there was a predominance of proteins in all the four microalgae species, a high lipid accumulation of $33 \pm 2\%$, was observed in *B. braunii* followed by lower lipid accumulation in *D. tertiolecta* (11.44 ± 1.8), *S. platensis* (11 ± 2.2), and *C. vulgaris* (9.95 ± 2.1). The other constituents in the microalgae were sugars, and pigments. The fixed CO_2 can be used by the microalgae to produce carbohydrates, lipids, proteins, and nucleic acids [195].

6.3. Anaerobic digestion and biogas production from microalgae

Anaerobic digestion is a versatile technology for the production of biogas from various industries and domestic wastes comprising of organic matter [208,209]. Microalgal biomass after lipid extraction comprises of proteins and carbohydrates that can be digested via anaerobic means to generate biogas, a renewable fuel. This can enhance the economic feasibility of the process of biodiesel synthesis. Sialve at al. [210] proposed anaerobic digestion of algal biomass after lipid extraction and envisaged that the energy generation can be more than that from the extracted lipid itself. The direct energy recovery by anaerobic digestion could also be more profitable when the algal lipid content in microalgae is lower than 40%. However, the direct energy recovery from microalgae has challenges viz. low biodegradability of microalgae (influenced by biochemical composition and nature of cell wall), high protein content in the cells of microalgae that releases ammonia and can be toxic, and presence of sodium in marine microalgal species that can be a hindrance in the performance of the anaerobic digester.

Mussgnug et al. [211] found that biogas generated from microalgae showed a higher methane generating capacity (7 to 13%) than from maize silage. The biogas produced by fermentation of microalgae, *Chlamydomonas reinhardtii* showed a high methane content and volatile gas solid of 66% and 587 mL (± 8.8 SE), respectively. Certain strains of microalgae were found to be detrimental to the bacterial community in the fermenter whereas, others showed their suitability. The detrimental effects was attributed to from the production of bacteriostatic or bactericidal compounds and indirect effects caused due to toxicity by high protein content and release of free ammonia. Alzate et al. [212] found that the substrate to inoculum ratios (*S/I*), biomass concentration, and pretreatment methods influences the biodegradability and methane production potential from the microalgae by anaerobic digestion. The *S/I* ratio and biomass concentration of 0.5 and 10 $\mathrm{g\ TS\ kg^{-1}}$, respectively, were found to be optimum for high biodegradability and methane production potential of microalgae.

Pretreatment of the microalgae by thermal hydrolysis enhanced the CH_4 productivity by 46–62%. The biological pretreatment resulted in negligible increase in CH_4 productivity. Ultrasound enhanced the productivity by 6 to 24% at 10,000 kJ kg^{-1} total solids. Ehimen et al. [213] investigated the pre-treatment routes of anaerobic digestion of filamentous algae, *Rhizoclonium*. It was observed that the blending of the biomass coupled with enzymatic pre-treatment improved the methane yield by more than 20% as compared to that from mechanical size reduction. Ehimen et al. [213] also been proposed the methane produced by anaerobic digestion of microalgae, glycerol, and wastewater for their utility in generation of electricity. The electricity could be used in various stages of production of microalgae (i.e. cultivation, dewatering, and extraction of oil) and the subsequent esterification/transesterification of microalgal oil.

One of the important aspects of biodiesel production is the generation of glycerol, a useful by-product. Glycerol formation is independent of the feedstock or catalyst used. Hence, an approach to use this product judiciously is of immense importance. Glycerol in its pure form has numerous applications in cosmetic, paint, automotive, food, pharmaceutical, pulp and paper, leather and textile industries. The glycerol that is derived as a co-product during the synthesis of biodiesel is impure and constitutes on 30% on volume basis. It can be concentrated and can be converted to high purity. However, its purification poses a challenge and its disposal too is economically and ecologically disadvantageous [214]. The valorization of glycerol along with the wastewater obtained after the treatment of biodiesel can be diverted to the production of methane. Siles et al. [215] reported anaerobic co-digestion of glycerol and wastewater derived from the biodiesel purification. The wastewater comprised of traces of biodiesel, oil, glycerol, free fatty acids, and methanol. The wastewater was initially demulsified and separated from oil and biodiesel by acidification with H_2SO_4 and centrifugation. The methane yield coefficient from the co-digestion of glycerol and wastewater has been reported to be 310 mL CH_4/g COD removal as compared to 292 mL CH_4/g COD removal from glycerol alone at 25 °C and 1 atm pressure. The efficiency of the process was approximately 81% thus indicating the COD removal was directly correlated to the methane production. The anaerobic digestion resulted in the production of 1.55 mL of CH_4/g VSS (volatile suspended solids) g COD h and an almost complete biodegradability of wastewater (100%) has been reported. Mussgnug et al. [216] utilized six microalgae species (freshwater and saltwater) as substrates for the production of biogas by anaerobic fermentation. The biogas production potential varied with the species and mode of pretreatment. It has been suggested that the microalgal species should be concentrated before fermentation but complete drying should be avoided as it decreases the biogas generation significantly. Table 10 depicts the biogas production from microalgae.

7. Emerging cost-effective methods for production of biodiesel

Considering the feedstock to be the major contributor in the total cost incurred in the synthesis process, the economics at present favors the waste cooking, waste frying, and waste animal and fish fats as feedstock for the production of biodiesel. Biodiesel obtained from used oil and fat has been found to be only marginally more expensive than the mineral diesel, whereas, that from microalgae is many times higher than mineral diesel [217]. The cost of biodiesel from *J. curcas* in India has been estimated to be US $ 0.87 per liter without taking in account the tax and profit margin [162]. The cost of algae derived biodiesel stands at US $ 1.25/lb as compared to US $ 0.43/lb for petroleum based diesel [40]. However, the availability of waste oil and fats is subject to various factors and its collection in an organized pattern has not been reported anywhere. This is followed by plant based oils which are available in substantial amount but finds other application too viz. soap manufacture, medicines etc. There are reports contrary to the general belief, that some *J. curcas* may need less water and fertilizers. They have been found to grow with optimum oil production only when supplied with sufficient nutrients and water. Their cultivation in the wastelands (including sodic land) and unutilized land (adjacent to the railway tracks as adopted in India) may offer an opportunity for a constant supply of feedstock oil. The economics at present places microalgal based feedstock at the bottom as they need technical expertise in cultivation, harvesting, and oil extraction which are cost intensive. Atadashi et al. [42] stated that simply using a low cost feedstock can reduce the cost of biodiesel by 25%. Thus, utilizing the non-edible and high acid value feedstocks viz. *P. pinnata*, *J. curcas*, castor oil, WCO, and animal fats have been advocated to bring down the production cost of biodiesel.

7.1. Microalgae cultivation

As microalgae have emerged as a potential feedstock for the synthesis of biodiesel, there have been several approaches to lowering the production cost of oil from microalgae. This section deals with the approaches that can be adopted to reduce the cost of biodiesel derived from the microalgal oil. Chiaramonti et al. [50] investigated the energy consumption and performance of conventional raceway pond with that of an innovative raceway pond. The traditional paddles were replaced with centrifugal pump (axial flow propeller) and an energy saving of 50% was estimated over an area 500 m^2 raceway pond. The innovative raceway pond was designed with a shallower depth (5 cm) for a low head of water in pond and increased microalgae concentration. The shallower depth offered reduction in volume of water by 75% and also facilitated an efficient downstream processing (separation of microalgae and water) as dewatering is considered to contribute a high overall cost. By the lowering the depth, the head loss in the pond curves was estimated to be lowered to around 7.5% as compared to the traditional design.

Photobioreactors have been continuously used in cultivation of microalgae as light, temperature, nitrogen starvation, and input of nutrients are better controlled, thus resulting in a high biomass productivity and lipid yield. Pegallapati et al. [218] emphasized that the energy input should be considered in addition to the biomass productivity per unit volume from the photobioreactor.

Table 10
Anaerobic digestion of microalgal biomass for biogas production.

Microalgae species	Country	Fresh (F)/saline (S) water	Biogas volatile solids (g VS^{-1})	CH_4 content (%)	Reference
Chlamydomonas reinhardtii (Green algae)	Germany	F	587 mL (±8.8)	66	[216]
Scenedesmus obliquus	Germany	F	287 mL (±10.1)	62	[216]
Mixture of *Chlamydomonas* (40%), *Scenesdesmus* (20%), *Nannochloropsis* (40%)	Spain	–	188–395 mL	70±3	[212]

VS = Volatile solids.

Hence, maximizing the net energy gain is of paramount importance when maximizing the biomass productivity. The approach should be to minimize the energy expense incurred at the various stages involved in microalgal culturing, harvesting and extraction of oil and synthesis of biodiesel. High power consumption is one of the major shortcomings of photobioreactor. The use of artificial light adds to the power consumption and increases capital costs [219]. The use of light emitting diodes has been proposed as a more cost effective method than the use of fluorescent light. Another emerging technology in optimization of biomass productivity of photobioreactors is the usage of light emitting diodes (LED) as they offer a narrow range of target wavelength. In an algal culture, using the LED based photobioreactor, the spectral output can be controlled and light intensity and frequency can be adjusted for the desired illumination [220]. It was found that *C. vulgaris* is able to adapt to light illumination with wavelength of 660 nm by altering its ratio of chlorophyll a to chlorophyll *b*. The biomass density obtained was 20 g L^{-1} dry cell weight (DCW) of culture medium and biomass productivity was measured to be 2.11 g L^{-1} DCW day^{-1}. Though, the present technique of the cultivation along with harvesting of microalgal oil and synthesis of biodiesel from microalgae are costly, the advancements in the techniques such as lowering the cost of LED in near future can lead to economic feasibility of the overall process.

The cost of closed systems is significantly higher than open ponds. Photobioreactor require 10 times the capital investment as compared to raceway ponds [221]. Reductive of the cost of construction of closed systems is imperative for the success of biodiesel production from microalgae [222]. It is possible to produce dewatered biomass at a cost ranging from \$5.08 to \$5.27 US kg^{-1} dry weight using tubular reactors [223,224]. Costs of mixing may account for a significant proportion of the total cost of biomass produced. These costs are approximately \$0.10, \$ 1.61 and \$ 3.93 US kg^{-1} for raceway, tubular reactors and flat panel reactors, respectively [225]. Reduction in the costs of mixing in photobioreactor will significantly improve the production economics. Raceway pond production estimates place the cost of dried algal biomass at \$0.34 US kg^{-1}. At a predicted lipid productivity of 24% lipid per gram DCW this translates to \$1.42 US kg^{-1} and \$209 US/bbl lipid. At a more favorable lipid yield of 40%, the cost decreases to \$0.85 US kg^{-1} and therefore \$126 US bbl^{-1} [226].

Considering the advantages and drawbacks of raceways and photobioreactors, the logical step in cost effective biomass production would be a combination of the technologies. Hybrid systems combine growth in bioreactors and raceway ponds [226]. Hybrid systems have been used historically in aquaculture for the growth of inoculum. This allows production of inoculum free of contamination and provides a large enough volume to give the culture of choice a competitive advantage in the open system [226]. The use of hybrid systems for biofuels production utilizes large scale photobioreactors and open ponds sequentially. The first stage of growth is undertaken within a bioreactor to maintain culture purity and achieve high biomass concentrations [219]. The second stage is undertaken in a raceway pond as this is ideal for nutrient stress. Hybrid systems can produce as much as 20–30 t ha^{-1} of lipid annually depend on climate favorability [222].

It has been reported by Acien et al. [60] that among the infrastructure needed in the synthesis of oil from the microalgae, photobioreactors offer possibilities where cost can be reduced by simplifying the design and the material used. Norsker et al. [225] estimated that the biomass production cost (including dewatering) by culturing in open pond, horizontal tubular photobioreactor, and flat panel photobioreactor were 4.95, 4.15, and 5.96 € kg^{-1}. The design of flat panel photobioreactor (closely spaced and vertical flat panel reactor) offers light dilution by larger specific surface and self-shading of the panels which results in a higher photosynthetic efficiency. However, the design of flat panel photobioreactor along with airflow, power efficiency and blower results in a comparatively higher cost. Acien et al. [60] utilized agricultural fertilizers instead of pure chemicals in the cultivation of microalgae which will lead to reduction in the production cost of biodiesel. Genetic modification has also been suggested by Chisti [186] for enhancement of photosynthetic efficiency, biomass growth rate, oil content, temperature tolerance (to reduce the expense of cooling); and to eliminate light saturation phenomenon, reduce photoinhibition (occurring in temperate and tropical area that reduces growth rate at midday light intensities) and susceptibility to photooxidation (that damages cells). Microalgae respond differently to photobioreactors as some algae are more sensitive to shear damage than the others. Some of the algae are also known to grow attached to the internal wall of the photobioreactor which prevents the penetration of light in the reactor and thus reduces the biomass productivity. Mutanda et al. [227] states that the quantity as well as quality of the oil and the ester content of the biodiesel derived from microalgae will determine the techno-economic feasibility of the technology. Razon and Tan [228] reported that the energy required for the cultivation of microalgae (*H. pluvialis* and *Nannochloropsis*) and its subsequent drying and cell disruption is a large energy input. Energy saving suggested has been the use of wastewater for culture of microalgae and recycling of some amount of algal biomass so that the use of photobioreactor, which again is cost intensive, is eliminated. Das and Obbard [229] studied the potential to reduce the energy supply consumed during mixing and as light imparted during culture of *Nannochloropsis*. It was projected through a model that by increasing the culture volume, 44% of the energy input imparted as constant supply could be saved through the incremental energy generated through the mixing of the microalgal culture in a photobioreactor.

The cost of conventional removal of nitrogen and phosphorus is reported to be \$4.4 kg^{-1} N and \$3.05 kg^{-1} P removed. Utilizing wastewater as a nutrient source for large scale can thus reduce the cost of wastewater treatment significantly. Zamalloa et al., [230] showed that a 70–110 t ha^{-1} $annum^{-1}$ facility using wastewater can result in a saving of \$48 400–\$74 800 ha^{-1} $annum^{-1}$ for nitrogen removal and \$4575–\$7625 ha^{-1} $annum^{-1}$ for phosphorus removal. The combination of saving from wastewater treatment and reduction of microalgae production costs is thus a win–win strategy when used for the production of energy or biofuels [231].

7.2. Harvesting, dewatering and extraction of lipids

Kim et al. [231] reported that the production of biodiesel and other products (excluding food supplements) from microalgae is not economically viable at present. Kim et al. [232] categorizes harvesting and dewatering as the most energy intensive steps and advocates for an efficient and improved method for the same to decrease the cost of microalgal oil production. They further devised an economical method using electro-coagulation coupled with flotation and termed it “continuous electrolytic microalgae harvest”. For the continuous cultivation and harvest of the microalgae, the technique exchanges the current direction (and thus the polarity exchange). Two distinct phases (one soluble and the other stable) are created during the harvest using a pair of electrodes. The first phase comprises of flocs of microalgal cells which are formed by destabilization of negative charged microalgae where metal ions are liberated from the dissolving electrode. In the second phase the metal ions generated from the first phase is terminated and bubbles generated from both electrodes renders the flocs to float. However, other electrochemical reactions may occur during the cultivation and harvesting due to polarity exchange that may cause damage to the cell. To make the oil

extraction economical and attractive, various approaches have been adopted by researchers. Dewatering is considered to account for 20 to 30% of the total cost of biofuels production [233]. Garg et al. [234] investigated the effectiveness of flotation on harvesting of marine microalgae and hydrophobicity of algae as an important parameter in the performance of flotation. A cationic collector, tetradecyl trimethylammonium bromide was found to improve the algal hydrophobicity and thus ease in the subsequent harvesting of algae. While utilizing *Chlorella* sp. and *Tetraselmis* sp., a marine microalga (grown in freshwater medium), for extraction of oil by flotation without using any collector. It was observed that in six minutes 93 and 6% of the oil was recoverable from former and the latter, respectively. This is attributed to the low hydrophobicity of *Tetraselmis* sp. as compared to *Chlorella* sp.

Rios et al. [235] stated that the harvesting of algal biomass is difficult due to their small size (3 to 30 μm and similarity in density with that of water). The conventional separation methods of microalgae from wastewater include sedimentation/flotation (with or without flocculation addition), centrifugation, and filtration. The technology for easier, faster, and more economical separation methods have been explored recently. Among the other methods, membrane filtration offers a technique which does not require addition of chemicals such as coagulants thus allowing the reuse of the nutrient laden water. The biomass can also be used directly to extract lipids. However, the technique involves energy input to maintain inter-membrane pressure difference and membranes have to be periodically replaced. Other difficulties encountered in membrane filtration include fouling due to the difference in shape, size, and compressibility of organic matter. Electrophoresis has developed as an important tool through which algae can be separated without use of chemicals. In the method, the algae move out of the solution under the influence of an electric field [205]. As harvesting and dewatering contributes a significant amount of total biomass production cost. Ahmad et al. [236] used crossflow microfiltration for harvesting *Chlorella* sp. suspensions (mean size diameter of approx. 3.67 μm) from the culture medium using a cellulose acetate membrane. Water is allowed to flow tangentially across the membrane in the crossflow microfiltration which allows the microalgal cells to remain in suspension thus limiting the cake formation. Vandamme et al. [237] demonstrated that the pre-concentration of microalgae via flocculation prior to dewatering (through centrifugation or filtration) can lower the energy needs of the process. However, algal organic matter excreted by microalgae interfered with the five flocculation methods that were tried viz. aluminum sulfate, chitosan, cationic starch, pH induced flocculation, and electro-coagulation–flocculation demonstrated different dose demands. When magnesium hydroxide was used as a flocculant, the dose was lowest (two fold) while that increased by nine fold when chitosan was used. For the rest of the methods (cationic starch, pH induced flocculation, and electro-coagulation–flocculation), the dosage increased five to six folds.

Efficient lipid extraction from microalgae involves a solvent extraction assisted with one of the processes viz. microwave, sonication or soxhlet. Irrespective of these processes, a solvent (dimethyl ether, chloroform, hexane, methanol, ethanol, or the mixture of the two) is used. Dimethyl ether is considered to be a safe solvent and is used for extraction in the production of food and food ingredients [78]. Osmotic shock (using NaCl) has also been used to disrupt the algal cells for lipid extraction. Cho et al. [238] stated that the best way of production of biodiesel is to extract the oil from de-watered microalgae by solvent extraction. However, if the cost is a constraint, wet extraction of oil from microalgal cell can be done to lower the extraction cost of lipids. Utilizing enzymatic hydrolysis, the cell wall of the microalgal sp. *C. vulgaris* was weakened by hydrolysis at 50 °C and pH 4.8 for 72 h. Hydrolysis of the microalgae by the enzymes, cellulases and β-glucosidases led to enhancement of the lipid extraction as compared to without hydrolysis with efficiency ranging from 29.2 to 73.1% depending on the solvent. The yield of FAAE also increased due to enzymatic hydrolysis, varying from 10.1 to 68.9% with the different types of solvent used. Using hexane as solvent, the FAME conversion was high but had a lower lipid extraction yield as only the hydrophobic portion was extracted. When chloroform–methanol was used as a solvent, the lipid extraction was enhanced due to extraction of both hydrophobic and hydrophilic content but the FAME conversion was low (67.8%). A high FAME productivity (97.3%) was obtained when the dry biomass was used which is the reason for the trend to dewater the algal biomass.

7.3. Low cost catalysts

The major cost involved in the synthesis of biodiesel is attributed to the feedstock and enzyme (if being used as catalyst). The cost attributed from the feedstock ranges from 70-95% of the total cost of biodiesel [57]. Among the type of catalysts, the cost factor at present favors the heterogeneous catalysts as it can be reused with minimal treatment. The enzymes used at present, though offers high specificity and selectivity for conversion of fatty acids to their respective alkyl esters are cost intensive. Whereas, the homogeneous catalysts offer low cost for the formation of FAAE. However, the residual homogeneous catalyst left in the product has to be purified by washing with water that generates colossal wastewater and hence limits its applicability at industrial level of production. Various heterogeneous based catalysts along with few homogeneous catalysts with high activity have been reported for the synthesis of biodiesel. Waste derived materials have been extensively utilized as solid catalysts in the production of biodiesel. These includes, waste chicken eggshell, mollusk (*Pila globosa*), clam shell (*Mereterix mereterix*), snail shell, waste capiz (*Amusium cristatum*), and waste fish (*Labeo rohita*) [116,239–244]. The waste chicken eggshell, mollusk, clam shell, snail shell, waste capiz comprises of calcium carbonate and their calcination (> 860 °C) result in the formation of calcium oxide which has high catalytic activity and has been utilized as solid base catalysts. The scales of the fish, *L. rohita* after calcination resulted in the formation of crystalline structure of β-Ca_3 $(PO_4)_2$. The β-tricalcium phosphate as solid catalyst resulted in a high yield (97.73%) of biodiesel [243]. The utilization of these waste materials as catalyst can lower the cost of biodiesel production and reduce their cost of disposal. These sources prominently comprise of calcium carbonate that upon calcination results in removal of carbon dioxide and formation of calcium oxide [245,246]. Water, if present causes saponification (with alkali catalyst) instead of fatty acid alkyl ester (i.e. biodiesel). Hence, any catalyst tolerant of water is preferred over those catalysts that are less tolerant to water. Few catalysts have been found to be resistant to small amounts of water. The mass production of microalgae is not currently economically feasible due to its various technological obstacles [247]. The cost if biodiesel derived from microalgae has to be lowered to at least at par with that obtained from the vegetable oil to make it marketable in the fuel industry.

7.4. Emerging extraction and conversion techniques

Emerging technologies in the synthesis of biodiesel includes application of microwave, membrane technology, and ultrasound. Microwave technology has gathered immense significance in extraction of oil from microalgal cell and the synthesis of biodiesel at a fast rate. Microwaves are electromagnetic waves with low energy content and belong to the category of non-ionizing radiations. These radiations influence molecular movements (ion

migration or dipole rotations) without changing the molecular structure. The polar solvents possessing the characteristics of low molecular weight and high dielectric constant when irradiated by microwave reaches their boiling point in a short time. The solvents that have been used for heating via microwave are water, methanol, ethanol, and acetone [248]. A study by Lee et al. [63] showed that the lipid extraction from microalgae (*Botryococcus* sp., *C. vulgaris*, *Scenedesmus* sp.) was more effective when using microwave in comparison to that from autoclaving, bead-beating, sonication, and osmotic shock. Manco et al. [248] who utilized microwave technology for production of biodiesel found the reaction time to be lowered to just 90 s using carborundum as pebbles.

Shuit et al. [249] utilized a membrane reactor for the synthesis of biodiesel. A membrane reactor combines reaction and separation in a single unit thus eliminating the intermediate processing steps. A membrane reactor also enhances the yield and selectivity of reactions. The concept behind the application of a membrane reactor is that when the transmembrane pressure is increased, methanol, biodiesel, glycerol, and catalyst will pass through and the unreacted and emulsified lipids are retained which will continue to react for the formation of biodiesel and glycerol. However, there can be certain limitations with the membrane technology as a good amount of methanol is also likely to pass through the membrane and may be a limiting factor in the synthesis process. Lohrey and Kochergin [250] modeled a project for co-location of an industry (cane sugar mill) and algae production for mutual benefits of both whereby the excess bagasse from sugar mill to be utilized for microalgal cultivation and the algae will utilize nutrients from the wastewater, thus a win–win scenario that leads to lowered overall production cost of biodiesel. The model projected 5.8 million L production of algal biodiesel per year utilizing the waste resources from cane sugar mill plant. On the estimate of at least 12% excess bagasse coming from the sugar mill, the algal farm is estimated to supply the entire fuel requirement through biodiesel for harvesting and transportation of sugarcane.

The transesterification reaction involves alcohol and oil that are much less miscible and hence stirring is required to overcome mass transfer limitations. Ultrasound is one of the techniques in which the reactants get emulsified thus reducing the catalyst requirement, reaction time and temperature. Another advantage is that the reaction becomes feasible with those feedstocks which otherwise are not conventionally suitable for transesterification [251]. Ultrasonic technology, thus offers an efficient technique for the production of biodiesel. Using power ultrasound (150 W), Ji et al. [252] reported a high yield and a short reaction time (10–20 min) for the synthesis of biodiesel with a high yield (~100%). High ultrasonic power (150 W) resulted in mixing of the reactants (oil and methanol) in a short time due to better mass transfer rate. However, a further increase in the ultrasonic power (200 W) reduced the yield of biodiesel. This has been attributed to formation of a mass of bubbles that reduce the methanol content in liquid phase and the interface area between the reactants. The overall reaction rate was found to be dependent on the extent of emulsification of the reaction system. Recently, Badday [253] utilized a solid acid catalyst (activated carbon supported heteropoly acid) for the transesterification of jatropha oil. The maximum yield of biodiesel obtained was 91% in 40 min at a high molar ratio of 25:1 at 65 °C. An advantage of using an ultrasonic mode of reaction was the generation of heat during collapse of the cavitation bubbles that were formed. The heat generated accounted for low input of external heat maintained at 56 °C for a cumulative heat to maintain the temperature of 65 °C.

A major challenge in the economical production of microalgae is as a result of the bioreactors being cost intensive. During the extraction of oil from microalgae, a substantial quantity is lost during the extraction process. To overcome these constrains, a low cost method has been envisaged by Origin Oil that breaks the cell with a low-power electromagnetic field using a process called as 'quantum fracturing'. CO_2 is added simultaneously to reduce the pH. The advantages of this method are: high efficiency of oil extraction in a single step without using chemicals, no requirement of initial dewatering and heavy machinery. The company, Origin Oil has further escalated the efficiency of oil extraction by 15% using a natural metabolite [51]. Tabernero et al. [51] proposed extraction of oil assuming almost total oil extraction from the cells of microalgae from 5000 m^2 of water surface to obtain 10,000 t of biodiesel utilizing lesser number of bioreactors thus lowering the overall production cost of biodiesel.

Gerde et al. [254] argued for optimization of the energy that is supplied during sonication to disrupt the cell of *Schizochytrium limacinum* and *C. reinhardtii*. As sonication is an efficient technique that does not require any solvent for oil extraction, the technique can be considered to be greener than the other techniques (solvent extraction). It was found that 800 J of energy per 10 mL of algal wet biomass for 25 min was sufficient for disruption of algal cell. When the sonication time was prolonged, free radicals were formed due to oxidation of arachidonic acid present in the oil of the microalgae. Pal and Prakash [255] devised a new methodology of transesterification in order to lower the cost of biodiesel from plant based oils. The technique was to disperse the lower amount of oil on alcohol with a small droplet size and the reaction was found to be feasible with low rate of stirring thus lowering the energy input in synthesis process. The gradual dispersion of the oil over alcohol provided a feasibility of high alcohol to oil molar ratio thus enhancing the forward rate of reaction. Jones and Mayfield [40] are of the view that economic biodiesel production could be achieved through combined biorefinery approach where multiple biofuels could be produced from one biomass source. Thus, microalgae and cyanobacteria could be used for the synthesis of biodiesel, bioethanol, biogas and biohydrogen.

7.5. Utilization of by-products

As the glycerol obtained after synthesis of biodiesel needs purification, their utilization for other valuable products has been envisaged by researchers. Chatzifragkou et al. [215] found that the crude glycerol obtained from the synthesis of biodiesel could be used as a carbon source and converted to valuable metabolic products (viz. organic acids, microbial biomass, single cell oil, mannitol) by using eukaryotic microorganisms (yeast and fungi). Nitayavardhana and Khanal [256] utilized glycerol derived from biodiesel to be utilized as an animal feed (an edible fungus, *Rhizopus microspores* var. *oligosporus*) through bioconversion. The main advantage of using glycerol as animal feed is a low cost of glycerol that is often discarded due to its energy intensive purification methods. Extraction of oil leaves the biomass as left over residues which still possess proteins and carbohydrates that could be further utilized. The residue can also be used as material for adsorption of dyes, heavy metals from the wastewater.

7.6. In-situ transesterification methods

The in-situ methods offer an advantage for merging two steps into one whereby the wet biomass is directly subjected to esterification/transesterification (thus skipping the dewatering and oil extraction step). Bypassing these two steps can minimize the cost by avoiding the use of large quantities of organic solvents and will make the biodiesel production greener [257]. The direct conversion of algal biomass to biodiesel via transesterification by using microwave irradiation has been reported by Patil et al. [59]. The use of the microwave technique has shown high conversion efficiency of oil in microalgal cells to biodiesel. It has been found to reduce the reaction time and the solvent volume when compared

with the separate process (of lipid extraction and transesterification) commonly adopted. A high power (1400 W) microwave was found to cause localized rapid superheating in the algal biomass and resulted in the rupture of cell walls of the microalgae with a high mass transfer rate and thus production of biodiesel. The maximum yield of FAAE obtained was 40.03% at optimized power (1400 W) which is quite low as per the minimum specifications of EN (> 96.5 wt%) and hence requires further improvement of the technology [59]. Wahlen et al. [257] demonstrated in-situ transesterification of pure strains of microalgae(*C. gracilis*), cyanobacteria, and wild mixed cultures (from wastewater lagoon) by a single step comprising of oil extraction for the synthesis of biodiesel and a high FAAE (max. 82%) was obtained. This was still lower than the EN 14214 specifications. However, the study also demonstrated that water played a crucial role in the yield of FAAE and a high yield of biodiesel approaching 100% has been reported which gradually lowered when the water content was increased with only 50% yield of FAAE at equal content of biomass and water. It was found that adding methanol in higher volume than that required for transesterification could offset the potential to reduce costs associated with drying the biomass. Ehimen et al. [258] reported that an increase in the alcohol volume and temperature was necessary to enhance the conversion of microalgal oil to FAAE during in-situ transesterification. Thus, the additional energy input in the removal of excess alcohol added during the reaction is a constraint and will off-set the advantage of the overall process.

Tran et al. [108] compared in-situ transesterification of *C. vulgaris* ESP-31 catalyzed by *Burkholderia* lipase to conventional transesterification of lipids extracted from same species. Wet microalgal biomass was subjected to sonication and then the lipid-containing slurry was mixed with hexane and transesterified by immobilized lipase. Transesterification of wet microalgal biomass showed higher biodiesel yield (97.3%) compared to transesterification of extracted lipids (72.1%). This approach eliminates drying, dewatering and extraction steps from the overall biodiesel production process and moreover gave good conversion and thus has great potential for application at large scale production. Kasim and Harvey [259] used 'in-situ' transesterification called 'reactive extraction' using the seeds of *J. curcas* for production of biodiesel. The particle size of the seeds that showed a high yield of biodiesel production was < 0.71 mm. The advantage of using 'in-situ' transesterification is that it negates contact of the parent oil with people. This is pertinent as the oil from *J. curcas* contains phorbol esters that poses health threat to people involved in the oil extraction. The major disadvantage of the process was a high requirement of methanol needed for transesterification which on optimization has been reported to be 400:1 (methanol to oil, molar ratio). The other parameters that influenced the reaction were in a reasonable limit (30 °C reaction temperature, 30 min reaction time, and 300 rpm reaction speed utilizing NaOH as catalyst). Su et al. [260] utilized dimethyl carbonate (DMC) and diethyl carbonate (DEC) as extraction solvent for oilseeds (*J. curcas*, and *Pistacia chinensis*) using the lipase Novozym 435 (*C. antarctica*). The solvents simultaneously worked as transesterification reagents for the extracted oil. The methyl and ethyl esters of *J. curcas* showed a high ester content of 95.9 and 94.5%, respectively. A comparatively lower methyl and ethyl esters content of 89.6 and 90.7%, respectively, were obtained with *P. chinensis* oilseeds. The solvent to seed ratio of 10:1 was found to give the optimum yield of alkyl esters. The optimum water content in oilseeds for *P. chinensis* has been reported to be 3.14 and 2.34% for DMC and DEC, respectively. While, for the *J. curcas* the optimum water content in oilseeds has been reported to be 3.02% for both the solvents.

The end product of the transesterification can be grouped into two categories: one fraction comprising of organic group and the other comprising of the aqueous group. The former includes fatty acid alkyl esters (biodiesel), free fatty acids, and (tri-, di-, and mono-) glycerides. The fraction comprising of the aqueous group are: glycerol, alcohol, and water. Crude glycerol, in addition to glycerol comprises of alcohol, water, soap, FAME, glycerides, FFA, and ash. Hu et al. [261] performed a study and found that glycerol along with methanol, FAME, soap, water accounted for > 85% of the mass of crude glycerol, whereas, glycerides, FFA, and ash accounted for < 15% of the total constituent. Jimenez et al. [262] reported that the method of enhancing the oxidation stability of the fuel is to remove the oxygen from the feedstock (stearic acid and tristearin) and to convert the lipids to hydrocarbons. Hydrocarbons have been better fuel properties as compared to biodiesel particularly in terms of oxidation stability, cold flow behavior, and compatibility in CI engines. The lipids were deoxygenated in a semi-batch autoclave using 20 wt% Ni/C, and 5 wt% Pd/C as catalyst. 5 wt% Pd/C showed a better selectivity in the formation of long chain hydrocarbons (particularly C17) as compared 20 wt% Ni/C which has been attributed to high acid strength and presence of acid sites in the latter resulting in adsorption of carbonaceous species on the catalytic surface and cracking reaction. However, 20 wt% Ni/C has been projected as an economical catalyst and other Ni based catalyst have been envisaged.

8. Energy return and greenhouse gas emission from biodiesel

The complete replacement of fossil fuels by biofuels does not look feasible at present. However, biofuels owing to its lower green house gas emissions (through carbon neutrality) and other harmful emissions (hydrocarbons, particulate matter, sulfur) can lead to a better sustainability by transition from current petroleum based society towards a greener biofuel based society [263]. The main factors that require consideration regarding justification of usage biodiesel is its energy return on energy invested (EROEI) and the benefits in terms of reduced exhaust emissions caused by the fuel. Producers tend to adopt those resources that provide a high EROEI value which fetch more profit [264]. As the current available feedstock for biodiesel (i.e. plant based) is scanty and the alternative feedstock (i.e. microalgae) is in development stages, a very high EROEI value is not expected from biodiesel. However, their environmental benefits (carbon sequestration and reduced toxic emissions) may justify their usage as biofuel. Thus, energy evaluation and assessment of greenhouse gas from the feedstock (microalgae and plant based) will determine the feasibility of the feedstock for synthesis of biodiesel. Batan et al. [265] proposed an industrial scale engineering model of *Nannochloropsis* species for its energy evaluation and assessment of greenhouse gases on combustion. Taking in account the energy consumption in terms of nutrients, diesel, and electricity at various stages viz. algal growth, dewatering, extraction of oil, conversion of oil to biodiesel, and transportation and distribution, the net energy ratio of biodiesel produced from *Nannochloropsis* was 0.93 MJ consumption per MJ of energy produced. The energy consumption for soybean oil was estimated to be substantially high with 1.64 MJ of energy consumption for production of 1 MJ of energy. The large difference of energy input between microalgae and soybean arose due to high cost value attributed to the soybean oil. Biodiesel developed from microalgae and soybean was found to reduce 75.29 and 71.73 g of CO_2-equivalent per MJ of energy produced which may justify the economical and environmental perspective in the usage of microalgae based fuel.

9. Plant seed oil and microalgal oil: A complementary to each other as biodiesel feedstock

The benefits of the use of biodiesel are enormous. Even as little as a 20% biodiesel blend with mineral diesel (i.e. B20) can reduce

CO_2 emissions by 15.7%. At a time when great concern worldwide with regard to the reduction of CO_2 emissions, the use of biodiesel as fuel seems quite relevant in the present perspective [266]. Doan et al. [75] advocates that the biodiversity of microalgae feedstocks will be important for the production of biodiesel. Biofuels from both terrestrial and microalgae biomass are constantly being upgraded for optimized oil yield and can be complementary to each other in providing a significant contribution towards the energy security for the present and near future [267]. Nevertheless, there are several more challenges in the utilization of microalgal oil for synthesis of biodiesel than those derived from plant oils. The limitation of plant oils is mainly due to its limited availability and alternative uses. A major challenge with microalgae is the extraction of oil with minimal energy input. Furthermore microalgae are difficult to manage and cannot survive the extreme conditions as several of the biofuels plants do. It has been found that changing the nutrient level does not always alter the composition of microalgae species. Under the conditions that can be achieved within the practical limitations, the lipid content can vary in the range 20–40% [267]. Another major challenge is the harvesting of microalgae. Huge ponds, lakes, and ocean shores offer a natural habitat for the culture of microalgae. However, the invasiveness of microalgae in the natural habitat can alter the course of ecosystem [268]. Temperature is a crucial factor in microalgae cultivation. Most of the species grow in a narrow range of variation in temperature with an upper limit of 35 °C baring a few exceptions. Species that are able to tolerate a high temperature include *D. tertiolecta* and *C. pyrenoidosa* having shown a temperature tolerance of 38.9 and 45.8 °C [269]. Grau et al. [270] advocates the use of small scale production of straight vegetable oil instead of large scale production for a self supply of biofuel to farmers.

There is no data available on the organic carbon uptake of plant (grown for biodiesel) from soil. This will not be an issue with algae as they are grown in aquatic environments. Among the prominent plant based feedstocks, *J. curcas* is known to grow under limited nutrient and water conditions. However, a wide gap is observed in potential and actual yield of this plant species. This has been attributed to the wild species of *J. curcas* which is commonly cultivated. This can be overcome by cross-pollination of *J. curcas* to get a hybrid variety with a high oil content and yield of the seed [64]. Similar to microalgae, the oil content and composition in plant seeds or kernels can vary too depending on the soil, nutrient conditions, and other biotic and abiotic factors. Achten et al. [271] stated that scientific evidences are yet to establish the claims of low water and nutrient tolerance, pest and disease resistance, and a high yield of *J. curcas*. The issues with microalgae and plant species include their water footprint. With microalgae species, there is a possibility of uncontrolled spread of algae causing algal blooms. Plant based feedstocks have the major issue of land versus fuel. The other significant issues include the organic carbon uptake from soil. Algal bloom is a common phenomenon in the nutrient rich conditions. Microalgae can grow indiscriminately if the nutrient control is not monitored and maintained. Uncontrolled microalgal growth may lead to enhanced productivity of biomass, and if grown in ocean may lead to degradation of coral habitat through several factors (reduced spatial heterogeneity by overgrowth, nighttime anoxic conditions).

Plant based feedstocks also have safety issues. *J. curcas* that was considered a wonder plant for application of its seed oil in the synthesis of biodiesel has been found to extract large amount of water from soil (a phenomenon similar with Eucalyptus). Though, *J. curcas* can survive in arid and semi-arid environment, its oil productivity is hampered when subjected to water stress. Hence, the water footprint for *J. curcas* will be high. Maes et al. [272] reported that *J. curcas* when subjected to water stress, combines C3-/CAM photosynthesis in succulent stems and the leaves shifts from C3-metabolism to more water efficient CAM. Johnston and Holloway [273] advocated for the development of biodiesel with a sustainable approach which can be achieved by commercialization of the efficient modes of production of biodiesel feedstocks (plants crops grown on marginal land and microalgae grown on aquatic environment) so that global supply of the food grains does not get affected. Hence, an appropriate crop (among the plant based and microalgae based) must be selected in that it is sustainable in providing long term availability of feedstock for production of biodiesel. *Moringa stenopetala* (plant oil seed) found in Ethiopia has been reported to possess a high oil content of (45 wt%) comprising primarily of mono-unsaturated fatty acid (78%) and saturated fatty acid (22%) [274]. Oil bearing plants that do not have alternative uses can be diverted for the production of biodiesel. As these sources are well located and there hardly exists any fluctuations in their harvest, they can also provide a good source of feedstock for the synthesis of biodiesel.

10. Conclusion

The need for a renewable fuel seems to have caught global attention. The prominence of biodiesel in transport sector is attributed to two important factors: to meet the escalating demand of liquid fuels and, pollution remediation due to combustion of biodiesel fuel in either neat form or blend form. Biodiesel, being renewable and environment friendly has been envisaged as a future fuel in the transport sector. The major constraint in the commercialization of biodiesel is its high cost as compared to mineral diesel which is contributed primarily by the feedstock. At present, plant based oil and microalgae have been projected as potential feedstocks for the production of biodiesel. There is still a long way to go for the plant and microalgal oil to cater the fulfillment of enormous amount of fuel demand and qualify to produce biodiesel of desired quality so as to meet the national/international specifications.

The progress in research work reported states that though the microalgae can be utilized as a potential feedstock for the synthesis of biodiesel, certain aspects have to be dealt meticulously to make the process more sustainable, green, and economical. As the lipid profile of microalgae is different from the plant oil, the conversion of the former to biodiesel has been found to vary considerably among the microalgal species. The fatty acid constituent also bears a significant effect on the fuel properties of biodiesel. In particular, the low temperature properties of biodiesel (cloud point, cold filter plugging point, and pour point) have an inverse relation with the oxidation stability of the fuel. The characterization of the microalgal and plant oil is an important parameter which has to be taken in account for the feasibility of the conversion of the contents to FAAE. Contrary to the plant based oils, the fatty acid alkyl ester (FAAE) obtained from the esterification/transesterification of microalgal oil derived from several oleaginous species at optimized conditions have not met the specification of European Nations (EN) 14214 which specifies a minimum content of 96.5%. This arises due to the presence of components other than fats and oils in the microalgal oil, which are not easily prone to esterification/transesterification.

Various methods have been proposed to reduce the cost of production of biodiesel using microalgal and plant oil. A simple and easy way to reduce the cost of biodiesel is by using a low cost feedstock. A blend of the feedstock can be effectively used as it provides desirable constituents for the synthesis of biodiesel. Bioreactors are one of the tools where modifications have been proposed for maximizing the oil output while cultivating microalgae. Emerging techniques for extraction and conversion using microwave, ultrasound, in-situ transesterification and low cost catalysts can make the biodiesel production process efficient.

The integration of biodiesel production with simultaneous production of methane via anaerobic digestion of glycerol and wastewater (obtained from purification of crude glycerol) can improve the economics and sustainability of process.

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